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Influence of dietary component manipulation and feed management strategies on growth and rumen development of weaned dairy heifers

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INFLUENCE OF DIETARY COMPONENT MANIPULATION AND FEED MANAGEMENT STRATEGIES ON GROWTH AND RUMEN DEVELOPMENT OF WEANED DAIRY HEIFERS.

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For my mom, who never doubted my abilities or dedication to learning and always encouraged independent thinking.

For my dad, who taught me that hard work was equal parts sweat on your brow, calluses on your hands, and passion to be better than average.

Thank you.

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LIST OF ABBREVIATIONS

AFC	Age at first calving (mo)
ADF	Acid detergent fiber
ADG	Average daily gain (kg/d)
ADGv	Average daily gain variance (kg/d)
A:P	Acetate-to-propionate ratio
BCS	Body condition score
BRSV	Bovine respiratory syncytial virus
BVD	Bovine viral diarrhea
BW	Body weight (kg)
BWv	Body weight variance (kg)
Ca	Calcium
CaD	Caudal dorsal
CaV	Caudal ventral
C:ADG	Feed cost per kg of average daily gain ($/kg)
Cl	Chloride
CONV	Conventional plane of milk replacer nutrition
CP	Crude protein
CP:ME	Ratio of crude protein to metabolizable energy (g/Mcal)

CrD	Cranial dorsal
CrV	Cranial ventral
Cu	Copper
DDGS	Dried distiller's grains plus solubles
DM	Dry matter
DMI	Dry matter intake (kg/d)
DNA	Deoxyribonucleic acid
ERW	Empty rumen weight
F:C	Forage-to-concentrate ratio
fNDF	Forage neutral detergent fiber
FPAC	Feldun Purdue Agricultural Center
G:F	Gain-to-feed ratio (feed efficiency)
H	Hydrogen
HCW	Hot carcass weight (kg)
HF	Hay feeder feed delivery treatment
HGC	Heart girth circumference (cm)
HH	Hip height (cm)
HI	High plane of milk replacer nutrition
HNFC	High non-fiber carbohydrate diet
HW	Hip width (cm)
IBR	Infectious bovine rhinotracheitis
K	Potassium
LNFC	Low non-fiber carbohydrate diet

LNFC+	Low non-fiber carbohydrate with added fat diet
LW	Live weight (kg)
MCP	Microbial crude protein
ME	Metabolizable energy (Mcal)
Mg	Magnesium
Mn	Manganese
MP	Metabolizable protein (g)
MR	Milk replacer
mRNA	Messenger ribonucleic acid
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fiber
NDFd	Neutral detergent fiber digestibility
NE_g	Net energy for gain (Mcal)
NE_m	Net energy for maintenance (Mcal)
NH3	Ammonia (mg/dL)
NRC	National Research Council
NFC	Non-fiber carbohydrate
NSC	Nonstructural carbohydrate
OM	Organic matter
P	Phosphorus
PACUC	Purdue Animal Care and Use Committee

PDREC	Purdue Dairy Research and Education Center
peNDF	Physically effective neutral detergent fiber
PI_3	Bovine parainfluenza virus type 3
Post	Post-weaning
Pre	Pre-weaning
PUN	Plasma urea nitrogen (mg/dL)
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
SARA	Subacute rumen acidosis
SBM	Soybean meal
SBS	Side-by-side feed delivery treatment
Se	Selenium
SIPAC	Southern Indiana Purdue Agricultural Center
TMR	Total mixed ration
TDN	Total digestible nutrients
USDA	United States Department of Agriculture
VFA	Volatile fatty acid
WH	Withers height (cm)
WW	Weaning weight (kg)
Zn	Zinc
20:80	20 percent forage, 80 percent concentrate diet
40:60	40 percent forage, 60 percent concentrate diet
60:40	60 percent forage, 40 percent concentrate diet

ABSTRACT

Dennis, Tana S. Ph.D., Purdue University, August 2016. Influence of Dietary Component Manipulation and Feed Management Strategies on Growth and Rumen Development of Weaned Dairy Heifers. Animal Sciences. Major Professors: Jon Schoonmaker and Tamilee Nennich.

Well-developed replacement heifers provide a central foundation for the continued success of the dairy industry. Emphasis on improving pre-weaned calf nutrition has predominated in the industry, but opportunities exist to improve post-weaning heifer nutrition and management. We aimed to evaluate common feed management strategies seen in the industry and their effects on growth, feed efficiency (G:F), and rumen development of calves from birth to 8 mo of age using pen- and individually-fed animal trials. Little information exists regarding post-weaning performance and rumen development of calves fed conventional or high planes of nutrition pre-weaning; therefore, we evaluated two milk replacer feeding programs with two post-weaning diets differing in non-fiber carbohydrate (NFC) content. Overall, calves fed a high plane of nutrition pre-weaning with a low NFC post-weaning diet were 9.8 kg and 12.4 kg lighter than calves fed low or high planes of nutrition pre-weaning, respectively, with a high NFC post-weaning diet. Additionally, average daily gain (ADG) and frame height were increased for high NFC-fed animals regardless of pre-

weaning treatment. Rumen development with respect to tissue morphology was similar between pre-weaning planes of nutrition in 12 wk old calves. However, 28 wk old animals previously fed a conventional milk replacer program had 20% greater papillae surface area compared to calves fed a higher plane of nutrition pre-weaning. Interestingly, rumen papillae morphology was similar between post-weaning diets and no interaction of pre- with post-weaning diet was observed. However, in a concurrent study evaluating low and high NFC diets using pen-fed heifers, feeding low NFC diets with added fat resulted in higher ADG and G:F compared to heifers fed high NFC diets despite similar dietary ME and CP content. Overall, these results suggest feeding diets with highly digestible carbohydrates to promote greater G:F and skeletal growth post-weaning, particularly when higher planes of nutrition are provided pre-weaning. As G:F improved when NFC and starch increased in the diet, we investigated increasing dietary concentrate proportions for growing heifers and the effects when switched to a high forage diet. Heifers fed 80% concentrate were the heaviest, tallest, and most feed efficient during the treatment period, but exhibited decreased in performance when switched to a high forage total mixed ration (TMR; 60% hay) compared to heifers previously fed 60% or 40% concentrate. Additionally, molar proportions of propionate and butyrate were greater when heifers were fed 80% or 60% concentrate, potentially influencing rumen development.

Delivering of feed as a TMR is common practice on dairy operations, as nutrients are delivered consistently with increased labor efficiency. Additionally, ensiled forages are commonly included as the primary forage component in heifer diets, although growth and intake responses when feeding ensiled forages as compared to hay are limited and

inconsistent. We compared feeding a common diet delivered by feeding hay and concentrate separately (HF), hay side-dressed with concentrate (SBS), and a TMR, observing that G:F of HF- and SBS-fed heifers was 8 to 10% greater overall compared to heifers fed a TMR. Additionally, HF-fed heifers were 13.5 kg heavier at the end of the study and had 5.6% greater DMI overall compared to SBS- and TMR-fed heifers. We also fed weaned heifers diets using mixed grass/legume forage preserved as hay or baleage as the only forage source to evaluate growth and efficiency. Heifers fed hay were 6.7 kg heavier than heifers fed baleage at the conclusion of the study. Heifers fed hay also consumed more DM and tended to have greater G:F than heifers fed baleage. As G:F was affected by forage preservation, feed delivery method, and carbohydrate inclusion in growing heifer diets, we postulated that rumen development may not be as mature as previously believed for heifers post-weaning. Greater understanding of the effects of feed management strategies on growing heifer performance has been achieved from our research, allowing more precise feeding recommendations and development of feeding programs to improve heifer development.

CHAPTER 1. REVIEW OF LITERATURE

1.1 Introduction

1.1.1 Overview of Replacement Heifer Development

Dairy replacement heifer development is an important enterprise that impacts the future productivity and sustainability of dairy operations. Success of a heifer development program relies heavily on feed management strategies from birth to first calving. As early as 50 yr ago, it was recognized that nutritional plane had an effect on growth and productive capacity of first-calf heifers (Crichton et al., 1959; Swanson, 1960). With improvements in genetics, nutrition, technology, and management since the 1960's, heifer feeding programs have changed to accommodate many of these variables. However, management changes were based on very limited heifer research or adult cattle data which may not reflect growing heifer biology.

In recent years, more complete understanding of the impact of nutrition during different growth phases has been established. Heifer development is generally categorized into phases by growth pre-weaning, growth before puberty, and growth post-puberty to calving. Pre-weaning and prepubertal growth in dairy heifers has garnered more attention recently due to the observable influence each growth phase has on first lactation milk yield and lifetime productivity. Numerous studies and meta-analyses have illustrated the positive effects of improved nutrition and greater average daily gain

(ADG) pre-weaning (Moallem et al., 2010; Soberon et al., 2012; Margerison et al., 2013; Soberon and Van Amburgh, 2013) and prepubertal (Stelwagen and Grieve, 1992; Choi et al., 1997; Zanton and Heinrichs, 2005; Krpálková et al., 2014) on milk yield in the first and subsequent lactations. Additionally, Bach (2011) reported that heifers with greater ADG from 12 to 65 d of age (0.8 kg/d) were more likely to remain in the herd to the second lactation than calves with ADG 0.7 kg/d or less ($R^2 = 0.23$). However, milk yields are not always increased in the first lactation with greater ADG during the pre-weaning (Morrison et al., 2009; Terré et al., 2009; Kiezebrink et al., 2015) or prepubertal (Van Amburgh et al., 1998; Lammers et al., 1999a; Abeni et al., 2000; Radcliff et al., 2000) period. Therefore, understanding the influence of nutrition and growth during the rearing period is still needed given the variability in milk production responses observed in the literature.

Negative effects of high prepubertal ADG on milk yield in the first lactation have been attributed to reduced parenchymal development, as allometric mammary tissue growth is altered in favor of fat pad deposition when growth rates exceed 0.7 kg/d (Sejrsen and Purup, 1997). However, more recent data has attributed deleterious effects of high prepubertal ADG to excessive energy intake post-weaning, as feeding diets with greater protein:energy ratios with ADG in excess of 1.2 kg/d did not compromise mammary parenchymal development in prepubertal heifers between 100 and 325 kg of body weight (Whitlock et al., 2002; Davis Rincker et al., 2008a). This illustrates composition of gain (protein vs. fat) is more important for mammary gland development than the absolute growth rate. Brown et al. (2005a) observed 69% less parenchymal tissue and 63% less extra-parenchymal fat per 100 kg of body weight (BW) in mammary

glands of heifers fed low (0.4 kg/d ADG) compared to high (0.7 kg/d ADG) planes of nutrition from 2 to 8 wk of age. However, when fed for 0.4 vs 1.1 kg/d ADG from 8 to 14 wk of age, amounts of parenchymal tissue were similar but extra-parenchymal fat deposition increased 1.2-fold for heifers gaining 1.1 kg/d ADG (Brown et al., 2005a). Positive nutritional influences on mammary development may be restricted to the pre-weaning period, as more recent data has also shown 86% lesser parenchymal tissue and 83% lesser mammary fat pad for Holstein calves fed a conventional 20% CP, 20% fat milk replacer (MR) program compared to an enhanced 28% CP, 25% fat MR program (Geiger et al., 2015). However, the mechanism by which increased parenchymal growth and greater ADG pre-weaning results in the potential for increased lifetime milk yield is unclear.

Additionally, prepubertal ADG exceeding 0.8 kg/d from 90 to 320 kg of BW have resulted in age at first calving (AFC) less than 22 mo; however, BW at calving were lower and milk yield was reduced 5% compared to heifers gaining between 0.6 and 0.8 kg/d (Van Amburgh et al., 1998). This result was likely related to body composition at breeding, as heifers gaining 1.0 kg/d were 2.9 cm shorter at the hip and 0.4 units higher in body condition score (BCS) than heifers gaining 0.6 kg/d at similar BW (Van Amburgh et al., 1998). Zanton and Heinrichs (2005) analyzed eight studies looking at the milk yield response to prepubertal growth rates and found a quadratic response in milk yield to growth rates ranging from 0.6 kg/d to 1.1 kg/d and heifers gaining 0.8 kg/d maximized milk production in the first lactation. Much of the response in milk yield was related to BW at calving, as there was a tendency for BW at calving to increase with increasing growth rates (Zanton and Heinrichs, 2005). These results suggest growth rates

throughout the prepubertal period, and not just pre-weaning, have the potential to influence first lactation milk yield, though much of the influence is likely due to composition of gain from birth to puberty. Given the considerable variation in first lactation performance due to prepubertal growth rates (before and after weaning) observed in the literature, additional nutritional management strategies warrant exploration with respect to replacement heifer development.

1.1.2 Significance of Replacement Heifer Development to the Dairy Industry

Despite potential negative effects of increased growth rates, several benefits exist that are of economic importance to dairy producers. Consistent benefits cited in the literature supporting increased growth rates include reduced AFC and reduced total feed costs over the rearing period, as dairy heifers attain puberty sooner (Lammers et al., 1999a), are bred earlier (Brickell et al., 2009), and spend fewer non-productive days on feed before entering the milking herd. Effects of greater growth rates on reproduction in growing heifers are largely due to the correlation between BW and puberty, in addition to physiological age (Mosely et al., 1982; Patterson et al., 1992; Hoffman, 1997; Grings et al., 1999). Mourits et al. (1999) modelled the economic implications of prepubertal growth rates in Dutch Holstein heifers as it related to net returns during the first lactation and suggested 0.7 kg/d ADG in 6 mo old heifers increased expected profit compared to 0.9 and 1.1 kg/d growth rates. However, the default assumptions in their economic model restricted prepubertal ADG in favor of compensatory gain during gestation based on Dutch recommended growth rates and findings from Foldager and Sejrsen (1987) and Sejrsen and Purup (1997). As previously discussed, negative effects of excessive growth

rates before puberty on first lactation milk production are most likely due to high energy intake relative to protein intake which would alter body composition before puberty in favor of fat deposition. More recent analysis of the optimum rearing conditions for replacement heifers in Pennsylvania Holstein herds showed milk production in the first lactation was depressed for growth rates exceeding 0.9 kg/d before puberty (Mourits et al., 2000). The authors also determined, under the conditions of their analysis, the highest expected income per heifer was optimized for prepubertal growth rates of 0.9 kg/d and maximum achievable growth rates of 1.1 kg/d post-puberty (Mourits et al., 2000). Krpálková et al. (2014) reported higher lifetime production (through 3 lactations) for Holstein heifers gaining more than 0.85 kg/d before puberty and over the entire non-productive period despite a negative effect on milk yield during the first lactation. Collectively, feeding heifers for higher prepubertal and overall ADG before calving can positively affect expected income per heifer and lifetime performance.

1.2 Growth Patterns in Replacement Heifers

Growth patterns in young animals can be described in several ways, but are generally classified into body weight, skeletal, and composition of gain. The sequence in which tissue growth occurs begins prenatally with neural tissue followed by bone, muscle, and adipose tissue (Owens et al., 1993). From conception to maturity, animal growth exhibits a sigmoidal curve with the inflection of the curve occurring around puberty for weight and frame (Owens et al., 1993). Many factors affect growth patterns, and balance between dietary protein and energy is needed to satisfy allometric and isometric growth demands early in life. Protein demands for lean tissue growth The

interrelationship between dietary protein and energy can affect total nutrient usage and efficiency (Gabler and Heinrichs, 2003a) and can also influence body composition. Previous studies investigating accelerated feeding regimens in pre-weaning calves (Brown et al., 2005b) and compensatory growth in 6 mo old heifers (Barash et al., 1994) have shown confounding effects of energy requirements on growth and body composition. In order to better understand nutrient requirements for growing heifers, the effects of physiology on growth patterns for BW gain, frame, and body composition need to be addressed.

1.2.1 Body Weight

Physiological and sexual maturity in growing cattle is markedly related to BW, as puberty typically occurs at 55% of mature BW in dairy heifers (NRC, 2001) and is more dependent on BW than physiological age (Schillo et al., 1992). Kertz et al. (1998) reported that approximately 50% of BW gain in dairy heifers occurs before puberty. Additionally, Holstein heifers that reach at least 620 kg of BW prepartum (Hoffman, 1997) or 82% of mature BW postpartum (NRC, 2001) are considered optimally grown to minimize dystocia and maximize first lactation milk yield. Heinrichs and Heinrichs (2011) indicated BW at first calving, in addition to several calf-hood variables, had a positive significant effect on first lactation milk production on 21 commercial Holstein herds in Pennsylvania. However, Grummer et al. (1995) cautioned that increasing BW at first calving above 660 kg prepartum with a BCS of greater than 3.5 may predispose primiparous heifers to metabolic disease early in lactation without a benefit of increased milk production. Ensuring adequate BW at first calving is highly dependent on growth

rates during the rearing period, though higher rates of gain require more energy for growth at higher BW (NRC, 2001). Additionally, absolute protein deposition increases with higher growth rates, but deposition slows as BW increases (Geay, 1984). Therefore, net efficiency of energy and protein utilization decreases with age and increasing BW, particularly in large frame cattle (Geay, 1984). This suggests most BW gain in growing dairy heifers should be achieved earlier in the rearing period, particularly before puberty.

1.2.2 Frame

As mentioned previously, inflection of growth curves in cattle occur around puberty, mostly due to a physiological shift from self-accelerating to self-inhibiting frame growth (Owens et al., 1993). Kertz et al. (1998) indicated 75% of mature withers height is attained in Holstein heifers by 12 mo of age and rates of frame growth slow following puberty. Additionally, the most cost-effective frame growth occurs in the first 12 mo of life as feed cost per cm of withers height gain was over 6 times greater at 24 mo of age compared to 12 mo of age (Kertz et al., 1998). Heinrichs et al. (1992) determined close relationships existed ($R^2 > 0.95$) between BW and frame measurements (withers height, heart girth, hip width, and body length) in Holstein heifers from birth to approximately 27 mo of age. However, growth curves for BW and frame as predicted by Heinrichs et al. (1992) are different in shape and slope. Cue et al. (2012) reported raw BW (predicted from heart girth measurements) and withers heights for Holstein, Brown Swiss, and Ayrshire heifers from birth up to first calving and noted BW growth patterns were linear with no apparent asymptote before calving, but a Brody growth equation fit withers heights as the data was non-linear. Inflection of the withers height growth curve for

Holsteins also appeared to occur around 6 mo of age (Cue et al., 2012), similar to observations presented by Kertz et al. (1998) that illustrate frame growth slows starting around 6 mo of age. Growth curves may also shift according to plane of nutrition, as Iwaniuk et al. (2015) reported significant mean biases for BW (overestimated by 1.6 kg) and hip height (underestimated by 5.4 cm) of heifers fed higher planes of nutrition using prediction equations from Heinrichs et al. (1992) and Kertz et al. (1998). The authors noted these prediction equations were based on heifers fed standard diets which were likely lower in protein and energy compared to currently recommended diets (Iwaniuk et al., 2015). Data used by Cue et al. (2012) were from farm observations in Canada from 1993 to 2003, and the same issue with feeding heifers lower planes of nutrition may also be reflected in their dataset. This highlights the need for updated growth predictions for heifers fed for greater growth rates in weight and frame. Additionally, the ability to influence frame growth through nutrition may decline as heifers reach pubertal age and weight.

1.2.3 Body Composition

Though BW and frame size in growing heifers are important metrics, composition of gain during the rearing period may influence future productivity of heifers more than either BW or height independently (Hoffman, 1997). Fox et al. (1999) evaluated the relationship of stage of growth and ADG to body composition in dairy heifers using mathematical modelling and reported as BW and ADG increases (from 0.6 to 1.0 kg/d), energy content of gain increases and protein content of gain decreases in 200 to 650 kg heifers. The rate at which fat deposition occurs is much more rapid at higher ADG (1.0

kg/d) compared to lower ADG (0.6 kg/d) when BW is 250 kg or greater, though body fat content would be predicted to be nearly double (28.5 vs. 14.5% for 1.0 vs. 0.6 kg/d, respectively) at mature BW for heifers fed for higher ADG (Fox et al., 1999). Theoretically, increasing energy intake above maintenance without increasing protein intake results in protein synthesis limiting growth and excess energy intake being deposited as fat (Garrett, 1987). Increasing protein intake above maintenance to support greater ADG should, therefore, favorably influence body composition to lean tissue and frame growth as body fat would dilute less of the body content of water, protein, and ash at a given weight and age (Owens et al., 1993). Subsequent studies on body composition in prepubertal (pre- and post-weaning) dairy heifers have indicated that increasing the proportion of protein to energy results in higher growth. Brown et al. (2005b) observed that Holstein heifers fed high energy and protein diets pre- and post-weaning were heavier and taller at 14 wk of age compared to heifers fed lower energy and protein diets. However, composition of gain for heifers fed higher protein and energy diets was shifted toward a 69% increase in carcass fat (7.6 vs. 4.5%) with similar carcass protein and ash content at 14 wk of age compared to heifers fed a lower plane of nutrition (Brown et al., 2005b). More recent data from Davis Rincker et al. (2008b) reported similar patterns in carcass fat accretion to those observed by Brown et al. (2005b) when 11 to 23 wk old Holstein heifers were fed a high energy diet. High energy diets formulated for 1.2 kg/d ADG according to NRC (2001) were fed for 0, 3, 6, or 12 wk and resulted in 9^{th} to 11^{th} rib fat content increasing linearly from 7.3 to 14.4% at 23 wk of age (Davis Rincker et al., 2008b). Though animals in each of the previous studies differed in age, the same trend in body composition growth was observed when higher planes of nutrition were

fed. Several studies evaluating the proportion of crude protein (CP) to metabolizable energy (ME) have concluded that increasing this proportion results in more efficient BW gain in prepubertal Holstein heifers (Lammers and Heinrichs, 2000; Gabler and Heinrichs, 2003a), which may correspond to more lean tissue deposition as trends were observed for linear increases in hip and withers height with increasing CP:ME in the diet (Gabler and Heinrichs, 2003a). However, body composition was not directly measured in these studies and claims of increased lean tissue growth are hypothetical. Additionally, heifers were limit-fed to either 2.45% of BW (Lammers and Heinrichs, 2000) or to achieve 800 g/d of ADG (Gabler and Heinrichs, 2003a), which could affect lean tissue deposition compared to feeding heifers for ad libitum intake and greater growth rates.

1.2.4 Pre-weaning Growth and Body Composition

During the first 8 wk of life, dairy heifers are managed differently compared to other livestock in that most calves are removed from their dam and fed liquid feed individually in order to monitor intake and health. With respect to MR, several products exist with varying proportions of CP and fatty acids. Several studies (Diaz et al., 2001; Tikofsky et al., 2001; Blome et al., 2003; Brown et al., 2005b; Bartlett et al., 2006; Hill et al., 2008a) within the last 20 years have evaluated the effects of manipulating MR formulations and feed management on calf growth and body composition.

Diaz et al. (2001) compared body composition of pre-weaned dairy calves fed for three rates of gain on a 30% CP and 20% fat MR and observed fat deposition increased as growth rate increased. Protein content of BW gain decreased with increasing growth

rate, corresponding to an observed increase in fat content of calves fed the highest plane of nutrition (Diaz et al., 2001). As calves aged, energy content of gain increased for growth rates of 1.0 kg/d or 1.4 kg/d, but not for 0.5 kg/d ADG (Diaz et al., 2001). The authors also found that feed efficiency improved 16% when calves were fed for the highest growth rate compared to the lowest growth rate (Diaz et al., 2001). Brown et al. (2005b) investigated the effects of high (30% CP, 16% fat MR on DM basis fed at 2.0% of BW) and low (22% CP, 22% fat MR on DM basis fed at 1.1% of BW) planes of nutrition pre-weaning and immediately post-weaning on growth and body composition. At 8 wk of age, body composition was similar between treatments, despite an advantage in ADG and total DM intake for calves fed a high plane of nutrition (Brown et al., 2005b). Lack of treatment responses pre-weaning was likely attributed to low animal numbers for analysis in the previous study; however, providing a high plane of nutrition using a higher protein, lower fat MR appeared to be advantageous for increasing growth without increasing adiposity (Brown et al., 2005b). This is in contrast to Diaz et al. (2001), though MR formulas differed between the two studies by 4.0% units in fat and energy intakes would have differed. Additionally, calf starter was offered by Brown et al. (2005b) but not by Diaz et al. (2001), which could also explain discrepancies in body composition as well as BW gain as grain would be less digestible than MR. Gains in BW reported by Diaz et al. (2001) were also nearly double those observed by Brown et al. (2005b), which would account for differences in body composition responses observed in each study.

Despite previously described discrepancies in body composition when feeding calves for greater BW gains pre-weaning, greater proportions of protein to fat in MR

would generally result in less body fat accretion overall. Blome et al. (2003) reported increased protein retention with increasing levels of CP in MR from 16% to 26% CP for male Holstein calves. As growth rates increased from 0.4 to 0.6 kg/d from 2 to 8 wk of age, structural and lean tissue deposition increased in lieu of fat deposition (Blome et al., 2003). Bascom et al. (2007) observed similar responses in body composition for Jersey bull calves fed MR with greater proportions of protein to fat, as calves fed whole milk or a 27% CP, 33% fat MR achieved more carcass fat compared to calves fed a 29% CP, 16% fat MR at relatively similar DM intakes. Interestingly, plasma urea N (PUN) concentrations increased with increasing CP content in the study by Blome et al. (2003), which usually indicates decreased N efficiency (McIntyre, 1970). However, the authors reported PUN values less than 9 mg/dL (Blome et al., 2003), which is below the normal threshold of 10 to 12 mg/dL for growing cattle (Byers and Moxon, 1980). This may indicate that for lower CP MR formulations (below 26% CP), calf demands for protein are in excess of supply. Several studies have also exhibited advantages to feeding higher amounts of protein based on growth performance and digestibility estimates (Hill et al., 2009b; Raeth-Knight et al., 2009; Hill et al., 2010). During the early neonatal period, calves are in a stage of self-accelerating growth and greater dietary protein intake is required to maximize skeletal growth. Therefore, when compared to feeding a 20% CP, 20% fat MR at 10% of birth weight, which is a typical feeding program in North America for dairy calves, increasing the proportion of protein to fat in the liquid diet results in increased growth rates.

Bartlett et al. (2006) observed total gastrointestinal tract mass increased when Holstein calves were fed MR at a rate of 1.75% of BW compared to 1.25% of BW

without calf starter. However, Kristensen et al. (2007) reported a linear decrease in total stomach mass with increasing MR allowance from 3.1 to 8.3 kg/d (25% CP, 18% fat) with calf starter provision in 5 wk-old calves; this result was mostly due to a linear decrease in reticulorumen mass. Similarly, Hill et al. (2008a) showed lesser empty stomach mass (reticulorumen, omasum, and abomasum) as a percent of empty BW at 9 wk of age when Holstein heifers were fed a 27% CP, 28% fat MR compared to a 28% CP, 20% fat MR or a 20% CP, 21% fat MR. However, Silper et al. (2014) compared feeding 4 or 6 L/d of a 21% CP, 17% fat MR reconstituted to 12.5% DM and did not observe differences in rumen mass with increased MR allowance. Disagreement among trials are likely due to differences in age at harvest and solid feed provision, as the presence of solid feed contributes to chemical and physical growth of the reticulorumen (Baldwin et al., 2004). More data is needed to further understand the relationship of pre-weaning nutrition plane with gastrointestinal development.

1.2.5 Post-weaning to Puberty Growth and Body Composition

Little information exists for post-weaned dairy heifers through breeding with respect to body composition. Some studies have shown that increasing CP:ME concentrations increases ADG and feed efficiency in prepubertal dairy heifers (Lammers and Heinrichs, 2000; Gabler and Heinrichs, 2003a). However, high value is placed on dairy heifers as replacements and comparative slaughter studies require large numbers of animals to show significant differences; thus, body composition measurements for dairy heifers post-weaning are limited.

In the data available for dairy heifers, similar trends are seen in body composition compared to dairy steers and beef animals. In a study using 280 kg dairy heifers, the authors found that stair-step nutrition alternating from 15% below NRC requirements to 40% above NRC requirements resulted in 1.8 times greater protein efficiency and growth efficiency than heifers fed a control diet (Park et al., 1987). Petitclerc et al. (1984) evaluated two growth rates on body composition from 155 to 340 kg in prepubertal dairy heifers and found carcass water content (56.3% vs. 60.1%) was lower and fat percentage (25.2% vs. 20.3%) was higher for heifers fed to gain 1.0 kg/d compared to 0.7 kg/d ADG. Waldo et al. (1997) found similar effects when feeding prepubertal heifers (181 to 334 kg BW) for 1.0 and 0.7 kg/d ADG as heifers yielded lighter empty BW (280 kg vs. 287 kg) and lesser proportions of body fat (14.7% vs. 16.6%) when fed for 0.7 kg/d ADG. Discrepancy between the two studies in body composition may have been due to the proportion of dietary protein to energy in each study, as dietary CP ranged from 12.5 to 13.5% in Petitclerc et al. (1984), whereas CP ranged from 15.8 to 22.4% in Waldo et al. (1997). Overall, body composition from comparative slaughter shows feeding higher energy diets for greater ADG post-weaning result in more adipose deposition up to puberty, particularly if protein is a limiting nutrient in the diet.

Efficient nutrient utilization can reduce rearing costs for replacement heifers as more weight and frame gain can be achieved using less feed. However, protein requirements for growing ruminants have little value to growth if energy requirements are not first satisfied (Preston, 1966). Therefore, the relationship between dietary protein and energy can affect total nutrient usage and efficiency (Gabler and Heinrichs, 2003a), as described with comparative slaughter studies. Previous studies investigating

compensatory growth and accelerated feeding regimens have shown confounding effects of energy requirements on body composition (Ørskov, 1982; NRC, 2001). Chelikani et al. (2003), also using urea space, estimated body fat and body protein increased more for 8 to 12 mo-old heifers gaining 0.8 to 1.1 kg/d compared to heifers gaining 0.5 kg/d; however, diets fed to heifers differed in CP and ME content, with CP decreasing from 20.9 to 13.5% and ME decreasing from 2.62 to 2.28 Mcal/kg of DM as ADG decreased. One limitation using urea space as an estimate for body fat composition is the method was developed in beef research, and beef breeds typically carry more adipose tissue compared to Holsteins at similar BW (Owens et al., 1993). However, relative treatment differences still reflect the effect of increasing energy intake on body composition in prepubertal heifers. Radcliff et al. (1997) reported 50% greater carcass fat and 6% lesser carcass protein for heifers (120 d of age to onset of puberty) fed a high plane of nutrition (19.4% CP, 1.2 Mcal/kg NE_g) compared to a low plane of nutrition (16.3% CP, 0.6 Mcal/kg NE_g). However, given the initial age and BW of heifers used by Radcliff et al. (1997), growth from birth to 120 d of age was less than optimal (estimated at < 800 g/d) and may have influenced fat deposition in heifers fed a high plan of nutrition post-weaning. In order to better understand nutrient requirements for growing dairy heifers, dietary protein to energy ratios require further investigation.

Several studies have evaluated the responses of growing heifers to varied CP:ME ratios in order to maximize nutrient efficiency. Gabler and Heinrichs (2003) evaluated varying dietary CP:ME ratios in limit-fed diets on feed efficiency and structural growth in prepubertal dairy heifers (124 d of age). Feed efficiency and skeletal growth improved as dietary protein content increased, likely due to linear decreases in nonstructural

carbohydrate (NSC):rumen degradable protein (RDP) ratios in treatment diets, which improved degradable protein utilization (Gabler and Heinrichs, 2003). Whitlock et al. (2002) fed isocaloric diets containing CP:ME ratios of 48, 57, and 66 g/Mcal to heifers from 100 d of age to the onset of puberty. Proportions of carcass protein and fat were similar among treatments at puberty, likely due to similar ADG and DM intake observed throughout the trial (Whitlock et al., 2002). Others have observed a tendency to increase body protein and ash content with 2% added rumen undegradable protein (RUP) in heifers from 3 to 10 mo of age (Moallem et al., 2004b). Lack of body composition changes in response to increasing protein in the diet may have been due to study design in Whitlock et al. (2002), as pen replicates were low and some heifers became pubertal earlier than anticipated. This resulted in heifers being slaughtered at ages relative to puberty attainment (~46 d after first corpus luteum) instead of at 7.5 mo of age (Whitlock et al., 2002). Additionally, differences may have been detected earlier in the feeding period as mammary parenchymal development (mg of DNA/kg of BW) was greater for heifers fed 66 compared to 48 g CP/Mcal ME at 250 d of age, but not 280 or 310 d of age (Whitlock et al., 2002). Had heifers been slaughtered at a common age in lieu of age relative to puberty, results may have shown body composition differences.

Data reporting the effect of dietary protein to energy ratios on gastrointestinal growth is limited in weaned, prepubertal heifers. Moallem et al. (2004b) observed an increase from 2.9 to 3.3% of live BW for the reticulorumen of heifers fed diets with 2% supplemental RUP at 5 mo of age, but not 10 mo of age. However, most comparative slaughter studies determining body composition only report weights of total gut components (stomach, intestines, liver, kidneys, etc. collectively) and do not separate

gastrointestinal tract components. More data is needed describing growth of different organs in the gastrointestinal tract under different feeding programs for weaned heifers.

1.3 Rumen Development in the Growing Dairy Heifer

One of the most physiologically and metabolically stressful times in a dairy heifer's life occurs during the first few weeks post-natal. Much of the stress at this time involves the transition from liquid to solid feed associated with weaning in conjunction with multiple changes in gut development. Rumen development within the first months of life may affect the future production of a dairy cow and delays in development could result in delays in growth.

1.3.1 Pre-weaning Rumen Development

Rumen development has been extensively investigated in dairy calves since the 1950's. Much of the groundwork for feeding recommendations of pre-weaned calves with respect to rumen development were established by work conducted by Warner et al. (1956), Tamate et al. (1962), and Sutton et al. (1963). The rumen is essentially non-functional at birth but has the capacity to increase from 30 to 70% of the total gut volume up to the time of weaning (Warner et al., 1956). Much of the development that occurs during this time is both physical and metabolic (Baldwin et al., 2004) and highly dependent on the establishment of fermentation within the organ. As liquid feed is effectively shunted to the abomasum via the esophageal groove, fermentation needs to be established by the consumption of solid feed. The developing rumen has been shown to be affected by physical and chemical form of the solid diet (Coverdale et al., 2004;

Lesmeister and Heinrichs, 2004; 2005; Khan et al., 2008), which is also influential on solid feed intake.

Establishment of the rumen ecosystem in the young ruminant occurs rapidly after birth. Anaerobic bacteria establishment in the rumen occurred by 48 hr in suckling lambs (Chaucheyras-Durand and Fonty, 2002) and 72 hr in dairy bulls (Anderson et al., 1987); however, establishment likely occurs sooner as rumen fluid samples were not taken prior to 48 hr of age in either study. Early establishment of rumen bacteria predominately occurs by animal-to-animal contact, particularly when offspring is reared with the dam or after colostrum consumption (Malmuthuge and Griebel, 2015). Though cellulolytic and amylolytic bacteria are present as early as 12 hr postpartum (Malmuthuge and Griebel, 2015), considerable rumen fermentation does not occur until substrates are available.

Solid feed in the form of calf starter is typically offered within the first few days of life in order to encourage intake and rumen fermentation early in the neonatal period. Physical form can vary widely depending on regional feedstuffs, processing, and other factors, but most formulations include starches, sugars, and protein from cereal grains, oilseeds, and by-product feeds. Fermentable starches and fiber are utilized by newly established populations of microbiota to produce VFA, of which propionate and butyrate are stimulatory for chemical development of the rumen epithelium (Baldwin et al., 2004). Khan et al. (2008) reported corn- and wheat-based calf starters increased rumen papillae length, density, and rumen wall thickness compared to oat- and barley-based calf starters, illustrating that starch source can affect rumen development. Castells et al. (2013) observed increased rumen weight as a percent of the total gastrointestinal tract and increased papillae length for calves fed a pelleted starter without roughage provision

(alfalfa or oat hay). However, when feeding calf starter diets varying in starch concentration (35 vs. 11% of DM), Kosiorowska et al. (2011) did not detect differences in rumen papillae morphology or rumen weight. The effects of starch and fiber in calf diets on rumen development has not been clearly defined by the current literature, as the studies investigating rumen development have varied in physical form (pelleted vs. textured starter), starch processing, roughage inclusion, or milk feeding level.

Increased starter intake is also associated with reductions in rumen pH, particularly with high starch starter formulations (Abdelgadir et al., 1996; Khan et al., 2008; Laarman et al., 2012b). Though reductions in pH below 5.8 can be problematic in mature cows, it is unclear whether low rumen pH in pre-ruminant calves causes metabolic stress or has negative effects on rumen development. Yohe et al. (2015) fed an extract of *Aspergillus oryzae*, a prebiotic that may increase populations of lactate-utilizing bacteria (*Megasphaera elsdenii*) in the rumen, to calves pre-weaning to potentially reduce concentrations of lactate and increase rumen pH. No differences in rumen pH or rumen tissue development were observed in response to prebiotic treatment; however, rumen pH increased from 5.6 to 6.2 and rumen epithelium and submucosa weights increased from 4 to 8 wk of age (Yohe et al., 2015). Yohe et al. (2015) did not report chemical composition of calf starter used in their study, but did describe the physical form as texturized which could allow calves to initiate rumination and help buffer the rumen, thereby increasing rumen pH. Porter et al. (2007) reported rumination was initiated 2 wk sooner and starter intake was greater for calves fed a coarse diet compared to a completely pelleted diet. However, rumen pH at 8 wk of age was statistically similar between starter forms, but numerically greater for calves fed a coarse

diet (5.0 vs. 5.4 for pelleted vs. coarse diet, respectively; Porter et al., 2007). Discrepancies in the literature regarding rumen pH responses to increased starter intake are likely influenced by several additional factors, and the relationship of rumen pH with rumen development is still undefined.

Solid feed intake and rumen development is also influenced by the liquid feeding program in growing calves. Kristensen et al. (2007) reported wet weights of the reticulorumen of 5 wk-old calves declined with increasing milk allowance, despite similar rumen epithelial morphology between calves fed 3.1, 4.8, 6.6, and 8.3 kg of MR per d at 12.3% DM dilution rate. These results corresponded to reductions in calf starter intake with increasing MR allowance (Kristensen et al., 2007), which may indicate more physical than chemical rumen development. Kosiorowska et al. (2011) observed similar responses to Kristensen et al. (2007) in rumen weights with increasing whole milk allowance; however, Silper et al. (2014) did not detect differences in rumen epithelium thickness and papillae length due to MR feeding strategy (4 vs 6 L/d of MR). Starter intake was similar between MR allowances prior to weaning at 60 d of age (Silper et al., 2014) which may partially explain lack of differences in rumen papillae morphology. However, mitotic indices on papillae were greater for calves receiving 6 compared to 4 L/d (Silper et al., 2014). The rumen epithelium mitotic index, expressed as the ratio of mitotic basal cell nuclei to total basal cell nuclei observed in a sample, can indicate cellular proliferative activity and is typically enhanced with greater intraruminal VFA concentrations (Sakata and Tamate, 1979; Baldwin et al., 2004) and insulin (Sakata et al., 1980). Though VFA concentrations were similar regardless of MR feeding program (Silper et al., 2014), increased nutrient availability with additional MR volume may have

stimulated cellular growth as insulin and glucose concentrations were elevated for calves fed 6 L/d. Davidson et al. (2013) also reported similar rumen morphology (papillae length and surface area) and rumen wall wet weights at 56 and 84 d of age when calves were previously fed a 20% CP, 20% fat MR at 454 g/d of DM compared to a 28% CP, 20% fat MR at 818 to 1,136 g/d of DM. However, in contrast to Silper et al. (2014), solid feed intake was greater for calves fed a lower plane of nutrition before initiating weaning at 45 d of age, but accumulated starter intakes were similar at 84 d of age between pre-weaning treatments (Davidson et al., 2013). These results suggest that reduced starter intake may not completely explain reductions in rumen development as defined by tissue morphology or reticulorumen weights. However, Khan et al. (2008) observed increased starter intake, reticulorumen weights, papillae length, and papillae density when calves were gradually weaned from whole milk fed at 20% of BW compared to a conventional milk feeding program (10% of BW until 49 d of age). Inconsistency in the data available may be partially explained by age at sampling relative to weaning, differences in protein and energy intake, proportion of CP to energy in the liquid diet, and starter nutrient composition and physical form. More data is needed evaluating different liquid and calf starter feeding programs and their effects on rumen development prior to weaning.

1.3.2 Post-weaning Rumen Development

Reduced digestibility coefficients for calves with reduced starter intakes, as was evident in work from Hill et al. (2009b), potentially reflects a reduction in rumen development, which would have significant effects on post-weaning performance. However, information is limited for rumen development post-weaning, despite an

acknowledged difference in rumen volume from weaning to maturity. The reticulorumen increases in volume from 30% to nearly 70% of the total foregut volume from birth to weaning (Warner et al., 1956), yet weaned calves typically experience reduced growth rates and intake when fed forages and high-fiber feed sources (Jahn et al., 1970; Hill et al., 2008b) generally utilized in mature ruminant diets. It also stands to reason that following weaning, there is some capacity for continued rumen development in response to increased energy intake from highly digestible carbohydrates.

McLeod and Baldwin (2000) illustrated increases in rumen and small intestinal mass were achieved in response to increased energy intake when dietary ME was increased using a high concentrate diet compared to a high forage diet in weaned lambs. In kids weaned at 28 d of age, protein, energy, or protein and energy restriction for 6 wk after weaning significantly arrested rumen and small intestinal development with respect to total weight (rumen) and length (small intestine), papillae morphology, and protein concentrations in rumen epithelial tissue compared to kids fed diets adequate in protein and energy (Sun et al., 2013). Following a 9 wk realimentation period, nutrient-restricted kids exhibited compensatory responses to adequate protein and energy intake in papillae morphology and protein concentrations in rumen epithelial tissue but never achieved similar BW or rumen tissue weights to controls at 111 d of age (Sun et al., 2013). These data indicate rumen development continues following weaning and is sensitive to protein and energy content in the diet. Additionally, negative effects of nutrient restriction could have lasting impacts on rumen development and function despite compensatory growth, potentially affecting the ability to absorb nutrients later in life.

Diet form and carbohydrate inclusion could also affect rumen development as energy availability may be altered by particle size and would differ between starch and forage fiber carbohydrate sources. Davidson et al. (2012a) evaluated physical form of grower diets for 13 to 24 wk old Holstein steers and reported similar growth and physical rumen development; however, there was a tendency to reduce rumen papillae length in cranial ventral tissue samples for calves fed texturized compared to pelleted diets. Davidson et al. (2012b) also tested different hay types fed to 13 to 22 wk old Holstein steers and observed steers fed higher CP, lower NDF alfalfa hay exhibited greater papillae surface area in ventral tissue samples compared to steers fed lower CP, higher NDF grass hay. However, baseline slaughter data were not reported and rumen development may have been affected by previous plane of nutrition. From both of these trials, it appears that diet digestibility post-weaning and forage quality may play a role in morphological development of rumen tissue.

In addition to physical and morphological rumen development, understanding the evolution of metabolic capacity of the rumen epithelium following weaning is needed. Supplementation with 2-methylbutyrate in the post-weaning diet (0, 3, 6, or 9 g/d of 2-methylbutyrate in concentrate) linearly increased BW, rumen weight, and proportion of total stomach weight (rumen, reticulum, omasum, and abomasum) to BW in 90 d-old calves weaned at 60 d of age, likely a function of increased concentrate intake both pre- and post-weaning (Liu et al., 2016). This data suggests that the capacity of the weaned calf rumen tissue to respond to butyrate continues post-weaning, as papillae length, width, and mRNA expression of growth hormone receptor and 3-hydroxy-3-methylglutaryl CoA synthase 1, a ketogenic enzyme associated with butyrate metabolism,

also increased linearly with increasing 2-methylbutyrate supplementation (Liu et al., 2016). While not explicitly compared, mRNA expression of 3-hydroxy-3-methylglutaryl CoA synthase 1 in the rumen epithelium numerically increased pre- to post-weaning independent of 2-methylbutyrate supplementation (Liu et al., 2016), illustrating continued capacity of the rumen tissue to mature metabolically after weaning. However, it is unclear if this trend continues beyond 30 d post-weaning in response to different diets or feed management strategies as few studies have evaluated gene expression in rumen epithelial tissue beyond the immediate post-weaning period.

1.4 Effects of Feed Management on Intake

1.4.1 Feed Intake Regulation

Several factors govern feed intake in ruminants, though factors are often categorized as either chemical or physical. Intake regulation depends on the chemical composition of the diet offered, as fermentation characteristics and carbohydrates will affect intake disparately. Ultimately, energy requirements for maintenance and production drive feed intake and are regulated both independently and mutually (Conrad, 1966). However, the physical capacity of the animal can also limit intake regardless of energy demands.

1.4.1.1 Metabolic Factors

Metabolic intake regulation is partly governed by energy supply from the diet and animal energy demand. Metabolic fuels, including propionate, acetate, and absorbed glucose, can signal satiety responses in cattle (Allen, 2000). This means diet

fermentability plays a significant role in intake regulation as volatile fatty acid (VFA) presence in the rumen can chemostatically limit meal size. Some reports attribute chemostatic intake regulation to changes in osmolality from accumulation of VFA and other fermentation end-products (Carter and Grovum, 1990b) sensed by the rumen wall (Carter and Grovum, 1990a). In sheep, osmotic pressure in the rumen explained over 95% of the variation in liquid outflow rate and water absorption across the rumen wall (López et al., 1994). As osmotic pressure increased, VFA absorption decreased resulting in accumulation of acetate and reduced rumen pH (López et al., 1994). However, absorption of propionate was enhanced with increasing osmotic pressure (López et al., 1994), most likely related to reduced rumen pH (Dijkstra, 1994). Though feed intake was not evaluated by López et al. (1994), reduced liquid flow rates from the rumen could partially explain reduced feed intake often associated with accumulation of VFA as Bergen (1972) observed significantly lesser feed intake in sheep fed forage-based diets when rumen osmolality increased using sodium acetate or sodium chloride. Feeding high concentrate diets to cattle typically results in a shift to propionate production, which often results in reduced feed intake. Substantial support exists for the hypophagic effects of propionate on intake in adult dairy cattle (Simkins Jr. et al., 1965; Frobish and Davis, 1977; Oba and Allen, 2003b; Oba and Allen, 2003a), but gut peptides and hormones may also play a significant role in feed intake regulation particularly when high energy diets are offered (Allen, 2000; Choi et al., 2000; Bradford and Allen, 2007; Bradford et al., 2008). Cholecystokinin has been implicated in reducing feed intake as increased plasma cholecystokinin concentrations were associated with reduced DM intakes in response to feeding high concentrations of total (Choi et al., 2000) and unsaturated fatty acids

(Bradford et al., 2008) in lactating cows. Additionally, plasma insulin has been observed to predict DM intake responses in lactating cows switched to a diet with greater starch fermentability, as greater circulating insulin before a diet change was associated with greater reductions in DM intake after a diet change (Bradford and Allen, 2007). However, preliminary circulating insulin only explained 28% of the variance in DM intake reduction after a diet change (Bradford and Allen, 2007) and other metabolic controls likely play a larger role in regulating intake compared to endocrine responses. Allen et al. (2005) suggested an additive effect of physical and metabolic regulation exists in cows, as inert rumen distension and VFA (acetate and propionate) infusion into the rumen was shown to depress intake in lactating cows fed hay- and silage-based diets (Mbanya et al., 1993). Additionally, chemical composition of diets can influence metabolic and physical factors regulating intake.

1.4.1.2 Chemical Factors

As energy supply and demand dictates intake to a large extent, it is important to understand how chemical composition and sources of energy differentially influence intake. Inclusion of supplemental fat increases the energy density of diets, which would be advantageous for increasing dairy heifer growth rates before puberty. However, intake is often depressed when large inclusion rates of supplemental fat are fed. While the mechanism by which fat suppresses DM intake in cattle remains poorly understood, it is thought that reduced fiber digestibility in the rumen (Jenkins and Palmquist, 1984), signaling of gut hormones responsible for satiety (Choi et al., 2000; Relling and Reynolds, 2007), and fatty acid oxidation in the liver (Allen et al., 2009) may play a role

in more mature ruminants. When soybean oil was included in calf starter at a rate of 20% of DM, starter intake was depressed for Holstein calves from 4 to 28 wk of age (Thibault et al., 2003) which also resulted in a reduction in ADG. This may be due to both starter palatability and fatty acid saturation, as plant oils tend to be high in mono- and polyunsaturated fatty acids. Degree of fatty acid saturation has also been shown to influence DM intake, as unsaturated fatty acids have been linked to reduced DM intake and smaller meal sizes in lactating dairy cows (Harvatine and Allen, 2006b). However, differences in intake were not observed in calves offered starter diets with partial replacement of animal fat with butyrate, coconut oil, and canola oil blends (Hill et al., 2007a). Additionally, increasing supplemental fat in feedlot diets from 0 to 8% of the diet DM using animal or blended vegetable fats resulted in linear improvements in feed conversion for crossbred steers without reducing DM intake (Zinn, 1989).

Including higher proportions of fermentable carbohydrates can also increase energy density of diets, as starches and sugars are more digestible sources of energy compared to fiber but are less likely to limit intake. Feeding high concentrate diets compared to high fiber diets improved DM digestibility in growing heifers (Reynolds et al., 1991; Moody et al., 2007; Lascano et al., 2009; Zanton and Heinrichs, 2009a) which presumably would allow for greater voluntary feed intake. Yang et al. (2001) observed lactating cows tended to consume 4% more DM (percent on BW) and 6% more OM (kg/d) when fed a 65% concentrate compared to a 45% concentrate diet containing barley and barley silage. Apparent digestibility of OM increased 7% and ruminal starch digestibility increased 22% when cows were fed a 65% concentrate diet, which would explain increased intake as a percent of BW. However, increased rumen degradable

starch has been shown to significantly reduce feed intake in other lactating cow studies (Allen, 2000). Disparity may be due to differences in diet composition across studies, effects on rumen osmolality, or fermentation acid production. Rotger et al. (2006b) observed a tendency to reduce DM intake in response to feeding barley- compared to corn-based diets for 130 kg dairy heifers. The authors also reported smaller meal size and greater rumination times for barley-based diets, which they identify as a mechanism to reduce acidosis in response to NSC fermentability. However, no differences in rumen pH were observed in response to dietary NSC (Rotger et al., 2006a), which suggests reductions in intake may have been related to overall energy intake or production of propionate, which has been shown to produce a hypophagic response in cows as described earlier.

Dietary protein has also been shown to influence DM intake of lactating cows, partly due to the relationship between RDP and feed digestibility (Oldham, 1984; Allen, 2000). However, information is limited for the effects of degradable fractions of CP on intake in growing heifers. Devant et al. (2000) evaluated high concentrate diets varying in protein degradability for growing Friesian crossbred heifers from 100 to 230 kg of BW and found no significant effect of protein degradability on DM intake, despite numerical increases in total tract DM digestibility. Tomlinson et al. (1997) investigated the influence of RUP from blood meal on intake and feed efficiency for 200 kg dairy heifers. Intake decreased linearly in response to increasing concentrations of RUP resulting in increased feed efficiency, which was attributed to an improvement in amino acid profile flowing to the small intestine (Tomlinson et al., 1997). Other studies have also shown increased intake and improved feed efficiency responses to RUP for growing heifers

(Casper et al., 1994; Bethard et al., 1997; Moallem et al., 2004a); however, several studies have shown no significant responses to RUP (Mäntysaari et al., 1989; Coomer et al., 1993; Whitlock et al., 2002), which may have been due to RUP source or energy content of the diets used in those studies. The effects of protein on intake appear to be highly dependent on dietary energy content.

1.4.1.3 Physical Factors

Physical feed intake regulation is fairly well-characterized in adult dairy cattle. Regulation is typically due to the distension effect of the diet in the reticulorumen coupled with increased feeding time needed for chewing (Allen, 2000). Increasing neutral detergent fiber (NDF) content in the diet also physically regulates intake as NDF, particularly from forage, ferments slower in the rumen compared to starch and sugar sources of energy. Waldo (1986) and Mertens (1994) suggested using NDF as the best chemical predictor of intake in ruminants, particularly when physical fill is limiting. Other sources of NDF, such as plant fiber by-products (soybean hulls, cottonseed hulls, etc.), may not have the same filling effect as forage NDF. Non-forage NDF sources have been shown to increase DM intake in lactating cows (Firkins, 1997; Grant, 1997) but not 24 mo-old heifers (Sarwar et al., 1991) or 8 to 12 wk-old calves (Hill et al., 2008b). Data on the effects of dietary NDF on growing heifers is limited, though Tomlinson et al. (1991) determined maximal DM intake per kg of $BW^{0.75}$ was achieved when feeding 182 kg dairy heifers forage-based diets with 41% NDF and decreased with increasing NDF. Additionally, Hoffman et al. (2008) concluded NDF intake was near-constant at 1.0% of BW in heifers from 163 to 643 kg of BW. If NDF intake is constant relative to BW in

growing heifers, this suggests that voluntary intake in heifers may be predominately restricted by physical fill.

Particle size can also physically influence intake as longer particles require more chewing to reduce particle size. When fed a total mixed ration (TMR) with short forage particle length or cottonseed hulls, early lactation dairy cows spent 2 to 13% less time chewing per kg of NDF intake compared to those fed a TMR with short forage particles or no forage dilution with cottonseed hulls (Kononoff and Heinrichs, 2003). Maulfair and Heinrichs (2013) observed a similar response to forage particle length with an increase in eating and total chewing time for mid-lactation cows fed corn silage-based TMR with long compared to short forage particle length. Dry matter intake increased from 29.4 to 31.4 kg/d when particle length distribution was reduced from 73 to 43% of corn silage particles larger than 8.98 mm (Maulfair and Heinrichs, 2013). Cows fed longer particle length diets also consumed more DM with particles greater than or equal to 18.0 mm (Maulfair and Heinrichs, 2013) which would require more time to reduce particle size to facilitate passage from the rumen. Yang and Beauchemin (2007) showed increased chewing time with greater forage particle length in 60% but not 35% forage diets when fed to post-peak lactating dairy cows. When feeding an all-forage diet, offering chopped compared to long-stem alfalfa hay increased DM intake nearly 6% in 340 kg dairy heifers, though DM and NDF digestibility was reduced when hay was chopped (Jaster and Murphy, 1983). Presumably, chopping hay may have increased passage rate, allowing for greater intake and reduced retention time in the rumen. Khan et al. (2014) recently reported DM intake tended to decrease as particle size distribution of particles > 19 mm increased from 60 to 72% of the diet for 200 kg heifers. Dietary

NDF was equal across treatments (52% on DM basis), therefore longer particle length in the diet likely physically restricted intake as total time eating increased 32 min/d for heifers fed diets with more long particles compared to short particles (Khan et al., 2014). However, forage inclusion in diets fed by Jaster and Murphy (1983) and Khan et al. (2014) were greater than 80% of the diet and other factors, including forage NDF and energy content, may have affected results. Currently, the effects of particle size on intake in heifers fed diets with lower forage inclusion is not defined.

Passage rate from the rumen would also be influenced by dietary fiber and particle size, potentially limiting intake if passage rate is reduced. Fractional passage rates of solid particles and NDF tended to increase with increasing DM intake for lactating cows fed a 74% forage diet compared to a 50% forage diet (Johnson and Combs, 1992). Mean retention time in the rumen was similar between cows fed 60% barley straw or no roughage (44.5 and 43.0 h, respectively), which was surprising given digestibility decreased with increasing barley straw inclusion from 20 to 60% of the diet (Bines and Davey, 1970). Total DM intake was least for cows receiving no roughage and greatest for cows receiving 60% barley straw (Bines and Davey, 1970), which could explain similar retention time as increased intake would increase passage rate from the rumen. Additionally, energy and protein were not balanced among treatment diets in that study, and other factors may have influenced retention time. Particle density also plays a role in passage rate and varies between NDF sources, with forages tending to be more buoyant than plant by-product sources of NDF (Grant, 1997). Nakamura and Owen (1989) showed when soybean hulls were included in lactating cow diets at rates of 25 to 48% of diet DM, passage rate of soybean hulls was roughly double that of alfalfa hay (10

vs. 5%/h, respectively). Dry matter intakes were similar to diets containing no soybean hulls (Nakamura and Owen, 1989), but soybean hulls and other non-forage fiber sources can increase DM intake in lactating cows up to 15% compared to control diets with at least 50% forage inclusion. When evaluated concurrently, particle size and density affected passage rate of inert particles through the digestive tract in 277 kg heifers fed only alfalfa hay (Ehle and Stern, 1986). Particles measuring 1.27 cm with 0.9 or 2.3 g/mL particle densities either were not recovered from the rumen (0.9 g/mL) or had a mean calculated retention time of 96 h (Ehle and Stern, 1986). Mean retention times were also greater than 90 h on average for particles measuring 0.3 cm with 0.9 or 2.3 g/mL particle densities (Ehle and Stern, 1986). Particles that were too light would likely remain in the rumen fiber mat and particles that were too heavy would settle in the rumen liquid fraction. Additionally, large particles would remain in the rumen until length was small enough to pass from the rumen. Older literature established that 1.18 mm was the critical particle length for feed to pass from the rumen; however, more recent data has shown that this threshold is greater than 1.18 mm particularly when DM intakes were high in lactating dairy cattle (Oshita et al., 2004; Maulfair et al., 2011). Interactions between NDF, particle size, and density play an important role in regulating intake, though observations in more mature cattle may not be similar to the effects of these factors in growing heifers.

1.4.1.4 Moisture and Fermented Feeds

Moisture content of the diet also has the potential to influence feed intake, particularly when fermented feeds are included in the diet. Including feeds with lower

DM content has merit, as there is less potential for diet sorting and greater flexibility in diet formulation using wetter forages and by-products feeds (Lahr et al., 1983). Thomas et al. (1961) compared diets for growing heifers containing alfalfa preserved as hay or silage and observed a 25.5% increase in DM intake for heifers fed hay from 5 to 12 mo of age. This also resulted in a 39.8% increase in ADG for heifers fed hay compared to silage (Thomas et al., 1961). Similar responses have been observed when water was added to identical diets for lactating cows, as Lahr et al. (1983) reported a linear increase in DM intake as dietary DM content increased from 40 to 78%. Estrada et al. (2004) also observed linearly increases in DM intake for lactating Holstein cows fed solely ryegrass as DM increased from 12.1 to 16.3% using controlled artificial drying. When fed diets containing 90% corn silage on a DM basis ad libitum, DM intake increased 2.4% for 250 kg beef heifers fed late-harvest (47.4% DM) compared to early-harvest (31.1% DM) corn silage (Worley et al., 1986). However, Merchen et al. (1986) observed that lambs and steers fed diets containing direct-cut silage, low-moisture silage, or hay had similar DM intakes. Nutrient dilution may influence intake with respect to reducing DM content in diets. As moisture increases in a given feed or diet, more total feed on an as-fed basis would be needed to meet required nutrient intakes on a DM basis. Rumen distension may be exceeded when wetter feed is consumed, restricting total available time needed to consume nutrients for maintenance and production (Allen, 2000). However, definitive evidence for the effects of dietary moisture on intake in dairy heifers is limited.

It is generally accepted that reduced DM intake associated with fermented feeds is largely due to fermentation products and not strictly moisture content. Fermented forages influence intake by sensory, physical, chemical, and metabolic mechanisms (Dulphy and

Demarquilly, 1994). Fermentation products in poorly-preserved forages, including volatile organic acids, alcohols, ammonia (NH3), and amines, can potentially reduce forage acceptability by the animal, thereby reducing voluntary intake (Thomas, 1961; Dulphy and Van Os, 1996; Allen, 2000). Additionally, fermentation products could increase osmolality in the rumen, which would also limit DM intake. In growing dairy heifers, feeding alfalfa silage resulted in reduced intakes and weight gain compared to feeding alfalfa hay (Thomas et al., 1961), although the mechanism by which intake and performance were depressed in growing heifers was not explained. More recently, Petit and Flipot (1992a) observed that steers fed an all-silage diet consumed less DM and exhibited improved feed conversion compared to steers fed an all-hay diet. Dennis et al. (2012) reported similar responses to Petit and Flipot (1992a) in DM intake and growth for prepubertal dairy heifers fed diets containing forage preserved as hay or wrapped baleage. The authors postulated that fiber digestibility may have been improved when forage was preserved as hay, as ADG per kg of NDF intake increased when heifers were fed the hay-based diet (Dennis et al., 2012). Given the limited body of data, inclusion of fermented feeds in prepubertal heifer diets should be discriminately considered as to not limit intake and growth at this age.

1.4.1.5 Management Factors

While diet composition and energy requirements dictate intake predominately, other management factors, including housing and feed presentation, can influence DM intake in cattle. Increasing stocking density in free housing can intensify competition for feed space, potentially inhibiting DM intake. DeVries and von Keyserlingk (2009a)

reported that increased competition for feed space reduced DM intake immediately after feed delivery in 234 kg BW heifers. Similarly in yearling dairy heifers, DM intake and growth decreased as linear feed space allowed per heifer decreased from 81 to 20 cm by increasing stocking density from 6 to 24 heifers per group; however, intake and growth was optimized with linear feed space allowance of 27 cm or 18 heifers per group (Keys et al., 1978). Authors described feeding management as restricted feeding, but details on feed delivery time or frequency were not provided. Presumably, linear feed space could be reduced with increased feeding frequency as less dominant animals would be given more opportunities to eat; however, Greter et al. (2013) reported for growing dairy heifers limit-fed to 2.0% of BW, increasing feeding frequency to twice per day did not affect feeding behavior compared to feeding once per d when heifers were allowed 40 or 29 cm of linear feed space per head. Robles et al. (2007) also reported no increase in DM intake when increasing feeding frequency from one to four times per d for 385 kg heifers fed for ad libitum intake; however, heifers in this study were tethered and not competing in a free housing system. In 2 to 4 mo-old Holstein calves, increasing feeding frequency from one to three times per d also did not increase DM intake when feeding a dry diet containing 95% concentrate and 5% chopped hay (Hill et al., 2015). In both of these studies, it is likely that linear feed space was not restrictive on intake and competition was low (Hill et al., 2015) or non-existent (Robles et al., 2007).

Feed delivery and presentation also affects voluntary intake. Quigley et al. (1992) observed that when 16 wk-old calves were offered grain and hay separately, DM intake was greater compared to when only grain was offered. However, when growing heifers (168 kg of BW) were offered a grass hay-based diet presented with components

separately, top-dressed, or as a TMR, DM intake was similar between feed presentation methods (DeVries and von Keyserlingk, 2009b). Heifers also sorted against particles 8 mm in length and larger when fed a top-dressed diet compared to a TMR (DeVries and von Keyserlingk, 2009b). Greter et al. (2010) observed similar results in intake and sorting behavior when heifers were fed a grass/alfalfa haylage-based diet presented as a top-dressed diet or a TMR. One prominent difference between these trials is the amount of roughage provided. Quigley et al. (1992) fed the equivalent of 12% roughage when calves were offered hay with grain, whereas DeVries and von Keyserlingk (2009b) and Greter et al. (2010) fed diets with approximately 65% of the diet from forage. This suggests that when forage inclusion is low or fed free-choice, DM intake will increase presumably to modulate the rumen environment; conversely, when forage inclusion is high, feeding components separately compared to a TMR would result in similar DM intake in growing heifers.

1.4.2 Feed Intake in Calves and Heifers

Factors influencing feed intake in adult cattle also apply to calves and heifers; however, the extent to which metabolic, chemical, or physical factors affect intake may differ given differences in energy requirements and physiology of growing heifers compared to adult cows. The chemical composition of the liquid diet, whether from whole milk or MR, has been shown to influence intake of calf starter, particularly with higher fat formulations and fat intakes (Hill et al., 2009b; Kertz and Loften, 2013). Additionally, increased feeding rates and enhanced nutrient profiles can also affect calf starter intake (Quigley et al., 2006; Kristensen et al., 2007). When Terré et al. (2007) fed

Holstein calves a 25% CP, 19% fat MR at 12.5% or 18.0% DM dilution rates, overall growth was improved but starter intake was depressed for calves fed the highest DM rate. Apparent digestibility of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), CP, and energy were also reduced for calves fed higher DM rates 1 wk post-weaning (Terré et al., 2007), which potentially influences post-weaning performance. Bach et al. (2013) observed similar responses in starter intake for pre-weaned calves fed 8 L/d of MR compared to 6 L/d of MR, as starter intake was reduced 52% for calves fed more MR before weaning. Reduced solid feed intake could potentially limit rumen development as rumen fermentation establishment can be delayed with greater liquid feeding rates.

With respect to fiber inclusion in growing calf diets, several recent studies have outlined the effects of roughage in the diet on intake. Terré et al. (2013) and Terré et al. (2015) identified that total DM intake in weaned calves increased when chopped roughage was offered with a low NDF pelleted calf starter compared to feeding a pelleted calf starter without roughage. Hill et al. (2009a) also found including 15% cottonseed hulls on a DM basis in calf starter increased voluntary intake 18% in Holstein calves compared to offering starter without cottonseed hulls. However, other studies have shown that as NDF or roughage increased in calf starter diet (Hill et al., 2008c), voluntary starter intake decreased. Differences may be due to physical form of the calf starter, as it appears pelleting or processing grains requires fibrous by-products and roughage in the diet, whereas offering textured starter with whole grains does not require additional fiber or roughage.

Offering wet and fermented feeds has also been shown to impact voluntary feed intake in young calves. Castells et al. (2012) tested several different forage sources (alfalfa hay, ryegrass hay, oat hay, barley straw, triticale silage, and corn silage) for pre-weaned dairy calves from 14 to 71 d of age fed a common calf starter (19.5% CP, 17.7% NDF) and 4 L/d of a 25% CP, 19% fat MR. Voluntary intake of the 2 silages offered were 47.5 to 55.8% lower than for calves offered oat hay and alfalfa hay, respectively, with other forage sources intermediate; however, total DM intake was similar, on average, for calves fed starter with either triticale silage or oat hay free-choice (Castells et al., 2012). Presumably, voluntary intake of forages separate from calf starter may have been affected by moisture, NDF content, or energy content, depending on forage type. Triticale silage, oat hay, corn silage, and alfalfa hay averaged 25, 91, 29, and 92% DM; 65, 60, 42, and 40% NDF; and 48, 101, 51, and 120 g/d forage DM intake, respectively (Castells et al., 2012). Feeding calves low DM, high NDF forage (triticale silage) resulted in the least voluntary forage intake but greatest total DM intake, whereas calves offered high DM, low NDF forage (alfalfa) consumed the most forage but averaged the lowest total DM intake (Castells et al., 2012). Additionally, intake of corn and triticale silage were lowest and similar, but total DM intake was numerically lower by 10% for calves fed corn silage (Castells et al., 2012). According to NRC (2001), corn silage provides about 26% more NE_m compared to triticale silage (1.57 vs. 1.25 Mcal/kg DM); however, energy concentrations for starter and forages were not provided by the authors, which could potentially explain disparate results in intake between silages and all forage sources. Overvest et al. (2016) evaluated feeding a silage-based TMR compared to 3 other dry feed diets to pre-weaned calves receiving up to 12 L/d of an acidified 26% CP,

16% fat MR. After weaning (49 d of age), calves offered a silage-based TMR consumed 35% less DM than calves previously offered a textured calf starter, starter and chopped hay fed separately, or a dry TMR containing 85% starter and 15% chopped hay (Overvest et al., 2016). The silage-based TMR averaged 51.5% DM while calf starter and chopped hay averaged 89.5% throughout their trial (Overvest et al., 2016). Despite as-fed intake of solid feeds being similar from pre-weaning through 12 wk of age (Overvest et al., 2016), DM intake was likely restricted by additional moisture in the silage-based TMR, particularly around weaning and immediately post-weaning. Factors that limit the ability of calves to meet energy requirements following removal of the liquid diet will have negative effects through the weaning process on growth.

For weaned calves, dietary energy and carbohydrates appear to have the greatest effect on voluntary intake. Tomlinson et al. (1991) studied the effects of varying dietary total digestible nutrients (TDN) and NDF on intake in heifers from 100 to 400 kg of BW. Second order polynomial equations of intake regressed against TDN, NDF, and acid-detergent fiber (ADF) best described the effect of diet on intake (Tomlinson et al., 1991). The authors determined that ad libitum intake was greatest with diets containing 70% TDN and 40% NDF and intake declined as TDN decreased with increasing NDF content. Independent of TDN, intake declined with NDF concentrations greater than 41% of the diet DM and ADF concentrations greater than 20% of the diet DM. Maximal intake was prevented by diluting TDN with structural carbohydrates, illustrating physical restriction by fill of the diet. However, as TDN increased above 70% in diets containing 40% NDF, intake decreased illustrating that even with low fill diets, intake may be differentially regulated by energy intake. Quigley et al. (1986) reported at dietary NDF concentrations

greater than 42% of DM, the proportion of NDF to ADF was positively correlated (r = 0.81) with DM intake in 100 to 400 kg heifers, whereas the relationship was less pronounced (r = 0.24) if dietary NDF was less than 42%. These results support differential regulation exists in growing heifers depending on structural carbohydrates in the diet, though other factors other than NDF have been shown to affect intake.

The interaction of dietary carbohydrates and CP fractions also affect intake in growing heifers. Casper et al. (1994) altered NSC and RUP in diets for 150 kg Holstein heifers and showed intakes tended to decrease as RUP decreased from 35 to 29% of CP content in the diet; however, the effect was likely driven by NSC source as intake with barley-based diets was different but not with corn-based diets. This response may reflect increased utilization of N associated with greater RUP intake from extruded SBM, as well as improved rumen synchrony of NSC and RDP, as a larger proportion of dietary CP was supplied from chopped alfalfa hay in barley diets, thereby providing greater proportions of RDP (Casper et al., 1994) and barley has been shown to have a faster rate of starch fermentation in the rumen compared to corn (Herrera-Saldana et al., 1990). Swartz et al. (1991) showed when 14 to 25 wk-old Holstein calves were fed isoenergetic and isonitrogenous diets ranging from 30 to 38% of CP as RUP, DM intake was greatest for calves fed diets containing 34% RUP and least for diets containing 30 and 38% RUP. Reduced intake at the greatest RUP concentration may have been related to blood meal inclusion to increase RUP, as blood meal is typically an unpalatable source of by-pass protein. Bethard et al. (1997) observed interactions of dietary energy with RUP on DM intake in 140 kg heifers, as feeding high energy diets with lower ADF content (67% TDN, 27% ADF) and low RUP (27% of CP) resulted in greatest DM intake compared to

other diets containing low energy and RUP (60% TDN, 34% RUP), low energy and high RUP (61% TDN, 50% RUP), and high energy and high RUP (66% TDN, 52% RUP). Overall, feed intake regulation appears to be multi-factorial and highly dependent on diet composition and animal physiology. However, understanding of how each of these factors affects feed intake in growing heifers requires further investigation.

1.5 Effects of Feed Management on Rumen Fermentation

Alteration of rumen fermentation can have an effect on feed efficiency and performance in ruminants. Microbial crude protein (MCP) production efficiency is highly dependent on the synchrony of substrates provided to the rumen bacteria (Herrera-Saldana et al., 1990; Firkins, 1996). That is, the rate of fermentation of both carbohydrates and proteins must be matched in the rumen to optimize MCP production efficiency. In addition to substrate synchrony, feed intake and diet digestibility are important factors governing rumen kinetics and passage rate of digesta which can affect MCP synthesis. However, there is an inverse relationship between feed intake and digestibility, with digestibility in the rumen decreasing as feed intake increases (Colucci et al., 1982). Impacts on digestibility are particularly prominent when high amounts of grain are fed, as intake typically increases with higher inclusions of grain in the diet. Therefore, digestibility and fermentation kinetics may influence feed efficiency and intake, which are important metrics to consider in feed management of prepubertal heifers.

1.5.1 Rumen Fermentation of Carbohydrates and Fats

Dietary carbohydrates contribute the largest proportion of energy-yielding products in ruminant diets, and usually are included at rates greater than 70% of the diet for dairy cattle (Nocek and Russell, 1988). Forages are often viewed as inexpensive sources of energy for ruminants; however, per Mcal of ME, starches, sugars, and fats are less expensive to feed as fiber digestion is energetically less favorable (VandeHaar and St-Pierre, 2006). Growing heifers are typically fed high-forage diets, which often results in reduced feed efficiency due to reduced digestibility of fiber in forage as compared to NSC. Replacing forages with highly digestible concentrates has been shown to increase feed efficiency (Zanton and Heinrichs, 2007) and organic matter (OM) and N digestibility (Zanton and Heinrichs, 2009a) when dairy heifers are precision-fed. Concentrate sources provide energy in the form of non-fiber carbohydrates (NFC) in the diet, which includes organic acids, sugars, starches, and neutral-detergent soluble fiber. Different fractions of NFC affect rumen fermentation in different ways, and will tend to influence rumen pH and microbial efficiency (Hall and Eastridge, 2014). Considering all sources of energy in a diet, several interactions occur that can also affect rumen fermentation.

Carbohydrate type can significantly affect rumen fermentation, as NDF and soluble fiber are predominately fermented to form acetate, starches to propionate, and sugars to butyrate (Wolin, 1974; Russell and Strobel, 1993). Increasing fermentable carbohydrates (starches and sugars) in the diet often reduces fiber digestibility as cellulolytic bacteria are sensitive to reduced rumen pH (Russell and Wilson, 1996). When comparing slowly and rapidly degradable starch in 20, 35, and 50% forage diets

for lactating cows, no interactions of degradable starch and forage:concentrate ratio were observed (Lechartier and Peyraud, 2010). As starch degradability increased and forage:concentrate ratio decreased, rumen pH and proportions of acetate decreased and propionate increased (Lechartier and Peyraud, 2010). Suárez et al. (2006) evaluated starter diets containing predominately pectin (beet pulp), non-forage NDF (1:1 corn grits and soybean hulls), and starch (1:1 corn and barley grain) to veal calves and found rumen fermentation shifted toward greater acetate with pectin and NDF, greater propionate with starch, and greater butyrate with pectin and starch. With respect to sugars, feeding di-saccharides (sucrose or lactose) to lactating cows increased proportions of butyrate and reduced rumen pH compared to feeding additional starch from rolled corn in barley silage-based diets (Gao and Oba, 2016). Surprisingly, few studies have evaluated rumen fermentation in calves fed sugars. Feeding granular sugar at 5% of DM reduced total VFA and acetate, but did not significantly affect rumen pH or butyrate concentrations compared to feeding a basal diet without sugar in calves up to 70 d of age (Beiranvand et al., 2014). When 12% molasses was included in calf starter, DM intake, ADG, and frame growth were reduced compared to feeding 5% molasses (Lesmeister and Heinrichs, 2005). Rumen papillae tended to be longer and wider with extra molasses in calf starter, but rumen fermentation characteristics were not reported (Lesmeister and Heinrichs, 2005). Presumably, butyrate increased in response to extra molasses, thereby nominally affecting papillae morphology; however, extra molasses impaired intake which may have resulted in lesser total VFA production. Most studies agree that reducing NDF in the diet will result in increased propionate concentrations at the expense of acetate (Zanton and Heinrichs, 2009b). Additionally, butyrate proportions are either unaffected or increased

when NDF is reduced, which would support continued rumen development. Therefore, formulating heifer diets with less forage and more NFC would favorably shift rumen fermentation toward propionate and butyrate production.

Total fat supplementation in diets can also affect rumen fermentation, with degree of fatty acid saturation explaining most of the variation in fermentation seen in the literature. As unsaturation increases in lactating cow diets, fiber digestibility in the rumen decreases (Pantoja et al., 1994; Pantoja et al., 1996). However, in the presence of calcium ions, unsaturated fatty acids can form insoluble salts in the rumen, thereby minimizing effects on rumen fermentation due to antagonistic interactions with cellulolytic bacteria (Palmquist and Jenkins, 1980). Harvatine and Allen (2006a) observed ruminal acetate increased and propionate decreased with increasing fatty acid unsaturation from calcium salts in corn silage-based diets. When weaned calves at 80 d of age were fed increasing concentrations of calcium salts of unsaturated fatty acids to replace starch from barley, proportions of acetate and branched-chain VFA increased and propionate and rumen pH decreased (Fallon et al., 1986). However, feed intake decreased up to 37% with the highest inclusion of fatty acids, resulting in nearly a 50% reduction in ADG immediately post-weaning (Fallon et al., 1986). In contrast, 190 kg BW Holstein calves fed 100% concentrate diets exhibited an opposite response to fatty acids, as acetate decreased and propionate increased when diets contained supplemental fat from soy oil compared to prilled, hydrogenated tallow (Bunting et al., 1996). Differences in rumen fermentation in response to fat are likely due to differences in unsaturated fatty acid source (calcium salt complexes vs. soy oil) and forage inclusion. While increasing acetate production is preferred to support milk fat production in

lactating cows, reduced propionate can limit gluconeogenesis in the growing heifer. Shifting rumen fermentation toward propionate production using saturated or rumen-inert fats has promise for improving performance; however, recent studies evaluating diets including supplemental fat for growing heifers are lacking.

1.5.2 Rumen Fermentation and Diet Particle Size

Recommendations for NDF content in the diet for adult dairy cattle is related to diet composition as well as physical effectiveness of the fiber to stimulate intake and maintain rumen health (Mertens, 1997). Physical effectiveness factors are components of a feed source that affect chewing time of the diet. Increased chewing and feeding time can result in increased saliva production per unit of feed (Beauchemin et al., 2008), thereby improving the buffering capacity in the rumen, maintaining rumen function (Mertens, 1997), and preventing sub-acute rumen acidosis (SARA) in adult dairy cattle (Stone, 2004). Bouts of SARA are defined as long periods of low rumen pH, typically under 5.8, that are associated with reduced fiber digestibility and altered rumen fermentation. Low pH levels are often observed when highly fermentable diets are fed, particularly with lactating cows. Increasing particle length and physical effectiveness of fiber in more mature ruminant diets for preserving rumen function is well-documented for lactating dairy cattle (Mertens, 1997; Maulfair et al., 2013). However, particle size and effective fiber content in weaned heifer diets is not well-defined with respect to rumen fermentation.

Beharka et al. (1998) compared particle size of calf starter diets with identical ingredient composition offered to Holstein calves fed whole milk at 8% of birth BW.

When fed ground diets, rumen pH was significantly lower at 4 and 6 wk of age but VFA concentrations were similar to calves fed an unground diet (Beharka et al., 1998). Additionally, calves fed ground diets also exhibited greater amylolytic bacteria counts at 6 and 8 wk of age compared to calves fed an unground diet (Beharka et al., 1998). Starter intake was equalized between treatments, which may explain similar VFA profiles as the chemical composition of the starter was identical. Reduced pH due to smaller particle size illustrates an increase in substrate availability, as BW at the end of the trial tended to be greater for calves fed the ground diet (Beharka et al., 1998). Suarez-Mena et al. (2016) observed when calves were fed a completely pelleted calf starter with increasing particle size of chopped straw (0.8 to 12.7 mm geometric mean particle size), rumen pH declined with age from 1 to 6 wk after starter was available (3 to 9 wk of age). However, Yohe et al. (2015) reported rumen pH increased from 4 to 8 wk of age for Holstein bull calves fed a texturized calf starter. Details regarding calf starter nutrient composition were not reported by Yohe et al. (2015), but increased particle size of a texturized starter compared to a pelleted starter could potentially influence pH as particle size of highly fermentable carbohydrates would likely be greater in a texturized starter, resulting in slower rates of fermentation and greater rumen pH. Other trials have also shown increased rumen pH with provision of chopped forages to pre-weaned calves fed pelleted starters (Castells et al., 2013; Terré et al., 2015). However, conflicting results in the literature demonstrates the need to better characterize the evolution of rumen fermentation in pre-weaned calves as well as the effects of intake and particle size on rumen fermentation in weaned heifers.

1.5.3 Rumen Fermentation and Diet Forage:Concentrate Ratio

In addition to intake, dietary proportions of grain and forage can have profound effects on diet digestibility and rumen fermentation. Dietary composition and fermentable OM intake affect total VFA production in the rumen and molar proportions of VFA, which are the primary energy sources for the ruminant animal. The VFA provide energy to the cow as precursors to lipogenesis (acetate), gluconeogenesis (propionate), and ketogenesis (butyrate). Additionally, increasing the proportion of fermentable fiber to fermentable OM typically increases the proportion of acetate to propionate (Aschenbach et al., 2011), as seen in pasture-based systems or high forage diets. When OM fermentability increases in ruminant diets, there is an observed increase in VFA production. Marked changes in rumen microbial populations occur during diet transitions, particularly from high- to low-forage diets as fermentation substrates are changed from mostly cellulose to starch and NSC (McAllister, 2000). When investigating changes in microbial profiles during a grain step-up diet regimen during grower/finisher phase, Fernando et al. (2010) observed that significant changes in microbial profiles occurred when beef steers changed from a 40 to 60% corn diet and from a 60 to 80% corn diet, but not from a 20 to 40% corn diet. The authors attribute this response to an increase in fermentable substrate available in the rumen (Fernando et al., 2010), yet there was not an observed effect increasing from 20 to 40% corn in the diet. What may explain the response is the slight decrease observed for populations of *Butyrivibrio fibrosolvens* going from 20 to 40% and 40 to 60% corn in the diet, followed by a 20-fold reduction in *B. fibrosolvens* population going from 60 to 80% corn in the diet (Fernando et al., 2010). This observation could be related to a drop in pH below the

optimal threshold for fibrolytic bacteria survival in the 80% corn diet. As OM fementability increases in the diet, molar proportions of acetate decrease and molar proportions of propionate increase in response to increased available substrates for amylolytic bacteria (Penner et al., 2011). Shifts in VFA concentrations due to changes in starch fermentability usually correspond to severe drops in rumen pH, which leads to acidosis in the rumen (Penner et al., 2011). Acidosis is an acute syndrome that occurs when diet fermentability, mostly due to starch, encourages accumulation of lactate in lieu of acetate, propionate, or butyrate. Lactate accumulation resulting in acidosis can affect microbial populations, as fibrolytic bacteria are extremely pH-sensitive and fibrolytic activity decreases at pH below 6.0 (Russell and Wilson, 1996). Consequently, if fibrolytic bacteria survival is low under high concentrate feeding conditions, it stands to reason that transitioning from low- to high-forage diets will result in lower fiber digestibility immediately after a change.

1.5.4 Rumen Fermentation and Feed Delivery

It has become common practice on commercial dairy operations to feed animals 6 mo of age and older using a TMR (DeVries and von Keyserlingk, 2009b). However, feed delivery methods for replacement heifers can vary between feeding dietary components separately and TMR delivery. Feed delivery using a TMR has been shown to reduce feed sorting behaviors against long particles in growing dairy heifers (Greter et al., 2010) and lactating cows (DeVries et al., 2007), which typically results in consistent rumen fermentation as nutrient supply is constant throughout the day. Consistent nutrient supply to the rumen can optimize rumen fermentation and microbial protein synthesis

(Nocek and Russell, 1988) and reduce susceptibility to drops in rumen pH due to rapid concentrate intake. Provision of a TMR compared to feeding dietary components separately has been shown to increase rumination time and saliva production in lactating cows consuming barley silage-based TMR diets (Maekawa et al., 2002), reducing the risk for rumen acidosis compared to feeding diet ingredients separately. However, when lactating Jerseys were fed concentrate separately from forage according to production level, milk production was significantly increased and feed costs were reduced compared to feeding a TMR (Gaynor et al., 1989). It is unclear if component feeding would have similar impacts on performance of growing heifers as those seen in adult dairy cattle.

1.6 Conclusions and Research Objectives

Replacement dairy heifers have been identified as an important investment to dairy producers, with feed costs comprising up to 70% of the total cost to raise a replacement heifer. Feed management strategies that improve growth rates and feed efficiency can potentially reduce feed costs from weaning to puberty. Feed management recommendations across the industry are limited for weaned dairy heifers until puberty. Developing feeding programs that maximize growth rates without over-conditioning prepubertal heifers are desirable. Manipulating diet composition in order to optimize feed intake and rumen fermentation for heifers at this age can potentially influence feed efficiency, particularly when higher concentrate diets are fed to produce fermentation profiles that may influence rumen development.

Information is limited for the effect of the pre-weaning diet on post-weaning performance and rumen development in replacement heifers, though previous studies

evaluating enhanced MR feeding programs have shown rumen development either is not negatively affected or is reduced when higher milk allowances or nutrient profiles are fed pre-weaning. Starter intakes are typically depressed when enhanced nutrition programs are employed, which would influence the physical and metabolic development of the rumen. Additionally, the effects of diet manipulation and feed management on rumen development post-weaning could impact growth and feed efficiency of replacement heifers. However, information is limited regarding recommended feeding strategies and diets that promote rumen development following weaning.

Therefore, the objectives of the research presented in this dissertation were to:

1. Identify potential interactions of pre-weaning and post-weaning nutrition on dairy calf performance, rumen fermentation parameters, and rumen development.
2. Evaluate the effects of diet composition on weaned, prepubertal dairy heifer growth, intake, efficiency, and rumen fermentation characteristics. Diet composition was manipulated by:
    a. Altering dietary carbohydrates and energy source (NFC vs. NDF; carbohydrate vs. fat)
    b. Increasing grain inclusion
    c. Forage preservation methods (hay vs. baled silage)
3. Evaluate the effects of feed delivery strategies on weaned, prepubertal dairy heifer growth, intake, efficiency, and rumen fermentation characteristics.

CHAPTER 2. EFFECTS OF PRE- AND POST-WEANING NUTRITION ON GROWTH, EFFICIENCY, AND RUMEN DEVELOPMENT OF DAIRY HEIFERS

2.1 Abstract

The objective of this study was to evaluate the interaction of pre-weaning and post-weaning nutrition on heifer performance, blood metabolites, and rumen fermentation. Holstein calves (43.5 ± 5.1 kg BW at birth; 39 heifers and 18 bulls) were assigned at 1 d of age to 1 of 4 treatments in a randomized complete block design with a 2×2 factorial arrangement of treatments. Pre-weaning milk replacer (MR) treatments were a 22% CP, 20% fat (as-fed basis) MR (CONV) or 28% CP, 20% fat MR (HI), with weaning based on starter intake (0.9 kg/d for 3 d on as-fed basis). Post-weaning treatments were low NFC (27% NFC on DM basis; LNFC) or high NFC (42% NFC; HNFC) grower diets fed individually for *ad libitum* intake from 12 to 28 wk of age. Weights, skeletal measurements, and blood were taken every 2 wk during the pre-weaning period. Post-weaning, BW were taken every 2 wk and skeletal measurements, blood, and rumen fluid were collected monthly. Pre- and post-weaning periods were analyzed separately and overall from birth to 28 wk. At weaning, calves fed HI were 15 d older, 18.0 kg heavier, and consumed 58% more DM through weaning compared to CONV; however, feed efficiency (G:F) was similar between HI and CONV from birth to weaning. From weaning to 11 wk, DMI was 53% greater for CONV; however, ADG from weaning

to 11 wk of age was similar, resulting in greater overall ADG from birth to 11 wk of age for HI. Hip height, hip width, and heart girth increased 2.7, 3.6, and 3.7%, respectively, for HI over CONV at 8 wk of age. Post-weaning, ADG was improved for HNFC, resulting in an 8.7 kg advantage in BW at 28 wk of age. Total DMI was similar between post-weaning treatments, and G:F was significantly improved for HNFC from 12 to 28 wk of age. Rumen fermentation and blood profiles were altered in favor of decreased acetate, increased butyrate, and reduced rumen NH_3 and plasma urea N for HNFC. Overall, calves fed HI+HNFC were 12.4 kg heavier at 28 wk compared to calves fed HI+LNFC, but similar in BW to calves fed CONV+HNFC. Rumen development with respect to tissue morphology was similar between pre- and post-weaning diets. Overall, our results suggest feeding diets with high NFC concentrations to promote greater growth rates, G:F, and skeletal growth immediately post-weaning, particularly when higher planes of nutrition are fed pre-weaning.

2.2 Introduction

Nutrient requirements of growing dairy heifers have received more attention over the last decade as the dairy industry has placed more emphasis on rearing healthy, well-developed replacement heifers before first calving. Several reviews have identified multiple factors related to heifer nutrition that impact the potential for future milk production, including pre-weaning growth rates (Soberon and Van Amburgh, 2013) and DMI at weaning (Heinrichs and Heinrichs, 2011). Increasing growth rates and feed intake usually increases feed costs, which are the largest cost of production for both lactating cows and heifer development (Heinrichs et al., 2013). Strategies to improve

feed efficiency and reduce heifer rearing costs warrant further exploration, as data is limited for growing heifers post-weaning to puberty.

Increased interest in feeding enhanced or high planes of nutrition to pre-weaned calves has occurred over the last decade in the dairy industry. Increasing total liquid volume fed (Khan et al., 2007; Sweeney et al., 2010; Silper et al., 2014), altering CP:fat ratios in the liquid diet (Cowles et al., 2006; Hill et al., 2006; Hill et al., 2008a; Hill et al., 2009b), or a combination of several feeding management strategies (Bartlett et al., 2006) have been evaluated for pre-weaned calves. A majority of studies agree that when compared to a 20% CP, 20% fat milk replacer (MR) fed at 10% of birth weight, increasing liquid feed allowance and increasing CP:fat ratios in the liquid diet results in increased growth rates. However, as more solids with enhanced nutrient profiles are being delivered in the liquid diet, calf starter intake is delayed (Quigley et al., 2006; Kristensen et al., 2007), often resulting in reduced rumen development (Baldwin et al., 2004).

Dietary carbohydrates contribute the largest proportion of energy-yielding products in ruminant diets, and usually are included at rates greater than 70% of the diet for dairy cattle (Nocek and Russell, 1988). Forages are often viewed as inexpensive sources of energy for ruminants; however, per Mcal of ME, starches, sugars, and fats are less expensive to feed as fiber digestion is energetically less favorable (VandeHaar and St-Pierre, 2006). Growing heifers are typically fed high forage diets, which often results in reduced feed efficiency (Zanton and Heinrichs, 2007; Lascano et al., 2009). Replacing forages with highly digestible concentrates has been shown to increase feed efficiency (Zanton and Heinrichs, 2007) and OM and N digestibility (Zanton and Heinrichs, 2009)

when dairy heifers are precision-fed to achieve similar ADG. Concentrate sources provide energy in the form of non-fiber carbohydrates (NFC) in the diet, which includes organic acids, sugars, starches, and neutral-detergent soluble fiber. Different fractions of NFC affect rumen fermentation in different ways, and will tend to influence rumen pH and microbial efficiency (Hall and Eastridge, 2014). Carbohydrate type can alter rumen fermentation, as NDF and soluble fiber are predominately metabolized to form acetate, starches to propionate, and sugars to butyrate (Wolin, 1974; Russell and Strobel, 1993). Altering rumen fermentation can have significant impacts on efficiency, as increased acetate:propionate ratios (A:P) have been associated with reduced metabolic efficiency and are typically observed when feeding high-fiber and high-forage diets (Zanton and Heinrichs, 2009).

In order to develop high-quality, efficient replacement heifers, early and optimal rumen development must occur. Rumen development in the calf is initiated when solid or liquid feeds are introduced into the reticulorumen and fermentation is established. Physical and metabolic development of the rumen is highly dependent on the presence of butyrate and propionate from the fermentation of solid feed (Baldwin et al., 2004). Increased concentrate feeding generally results in increased concentrations of propionate and butyrate, of which 30% to 70% and up to 80% to 90% of each volatile fatty acid (VFA), respectively, is utilized by the rumen epithelium as an energy substrate (Gäbel et al., 2002; Rémond et al., 2007). Manipulating rumen fermentation in favor of end products that promote rumen development can potentially improve growth and efficiency of prepubertal dairy heifers. Davidson et al. (2012a) evaluated physical form of grower diets for 13 to 24 wk old Holstein steers and reported similar growth and physical rumen

development; however, there was a tendency to reduce rumen papillae length in cranial ventral tissue samples for calves fed texturized compared to pelleted diets. Davidson et al. (2012b) also tested different hay types fed to 13 to 22 wk old Holstein steers and observed steers fed higher CP, lower NDF alfalfa hay exhibited greater papillae surface area in ventral tissue samples compared to steers fed lower CP, higher NDF grass hay. From both of these trials, it appears that diet fermentability, particle size, and forage quality may play a role in physical development of the rumen. The reticulorumen increases in volume from 30% to nearly 70% of the total foregut volume from birth to weaning (Warner et al., 1956), yet weaned calves typically experience reduced growth rates and intake when fed forages and high-fiber feed sources (Jahn et al., 1970; Hill et al., 2008c) generally utilized in mature ruminant diets. McLeod and Baldwin (2000) illustrated an increase in rumen and intestinal mass when ME intake was increased using a high concentrate diet compared to a high forage diet in weaned lambs. Additionally, McLeod et al. (2007) observed a 14.7% increase in rumen mass when 243 kg beef steers were fed 214 kcal ME/kg $BW^{0.75}$ per d compared to 161 kcal ME/kg $BW^{0.75}$ per d. The authors also reported an 18.9% increase in rumen mass when starch hydrolysate was infused ruminally compared to abomasally on the lesser energy diet to increase ME supply (McLeod et al., 2007), suggesting site of ME utilization plays a larger role in gut development than ME intake alone. It stands to reason that following weaning, there is some capacity for continued rumen development in response to increased ME intake from highly fermentable carbohydrates.

To date, few studies have evaluated the effects of the pre-weaning diet on post-weaning performance of dairy heifers. Those studies that do report post-weaning

responses generally report data from 2 wk to 8 wk (Jasper and Weary, 2002) (Hill et al., 2010) following weaning. Additionally, even fewer studies have evaluated potential interactions between pre-weaning and post-weaning nutrition on growth in prepubertal heifers. Therefore, the objectives of the current study were to evaluate the potential interactions of pre-weaning plane of nutrition and post-weaning dietary NFC on performance and rumen development of dairy heifers from birth to 28 wk of age.

2.3 Materials and Methods

2.3.1 Animals and Housing

This study was conducted at the Purdue Dairy Research and Education Center (PDREC) in West Lafayette, IN and Feldun Purdue Agricultural Center (FPAC) in Bedford, IN from March 2nd, 2013 to October 21st, 2014 using Holstein calves born at PDREC. All animal-related procedures were conducted in compliance with approved protocols from the Purdue Animal Care and Use Committee (PACUC no. 1304000843). Forty heifers and 18 bulls (43.5 ± 5.1 kg of BW at birth) were assigned at 1 d of age to pre- and post-weaning treatments and blocked by birth date. All calves received 1.9 L of fresh or thawed colostrum measuring at least 22% on a Brix scale using a digital refractometer (PA201; MISCO Refractometer, Cleveland, OH) within 4 h of birth and an additional 1.9 L within 24 h. Calves were vaccinated for *Clostridium perfringens* type A (Novartis Animal Health Inc., Larchwood, IA) and moved to individual calf hutches (Calf-Tel; Hampel Corp., Germantown, WI) within 3 d of age. Hutch dimensions were 1.5 m x 1.2 m of interior area with an exterior pen area of 1.4 m x 1.0 m. Hutches were bedded with pine shavings from March to October each year and straw from October to

March. Water and calf starter (Vita-Plus Corp., Madison, WI) were available for *ad libitum* intake inside calf hutches. All calves were disbudded using a butane dehorner with local lidocaine anesthetic and vaccinated for bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), bovine parainfluenza virus type 3 (PI_3), bovine respiratory syncytial virus (BRSV), and leptospirosis (Bovi-Shield Gold FP5 L5 HB; Zoetis Inc., Kalamazoo, MI) at approximately 4 wk of age and given booster injections at 8 wk of age. Following weaning, calves were grouped by pre-weaning treatment within block and fed in pairs until approximately 11 wk of age. Male calves that were transported to FPAC for the post-weaning grower period were castrated approximately 7 d following weaning; male calves used to evaluate rumen development remained at PDREC and were left intact in order to avoid stress associated with castration. Calves were vaccinated intra-nasally for PI_3 (INFORCE 3; Zoetis) 3 d prior to shipping to FPAC. At approximately 11 wk of age, all calves in a block were transported to FPAC for the post-weaning grower period and were individually housed until 28 wk of age. Calves were housed in pens located in a naturally-ventilated barn with 2.4 m x 1.8 m pens, 0.9 m of feeding space, and unrestricted access to water. Pens were covered by slanted steel roofing and bedded with straw throughout the study as needed. At 16 wk of age, calves were dewormed (Dectomax pour-on; Zoetis), and vaccinated for BVD, IBR, PI_3, BRSV, and leptospirosis (Bovi-Shield Gold FP5 L5 HB; Zoetis) and were given booster injections 4 wk following the first vaccination.

2.3.2 Experimental Design and Treatments

Calves were assigned to treatments in a randomized complete block design with a 2×2 factorial arrangement of treatments. Pre-weaning MR treatments were a 22% CP, 20% fat MR (as-fed basis; Amplifier Max, Land O'Lakes Animal Milk Products, Shoreview, MN; CONV) or 28% CP, 20% fat MR (Cow's Match, Land O'Lakes Animal Milk Products; HI). Calves fed CONV received 350 g of solids/feeding until 1 wk prior to weaning and calves fed HI received 380 g/feeding from d 1 to d 7 and 570 g/feeding from d 8 until 1 wk prior to weaning. All calves received CONV following colostrum feedings until moved to individual hutches before 3 d of age. Milk replacer was fed twice/d until 7 d prior to weaning, then once/d until complete weaning for both CONV and HI. All calves were fed texturized calf starter (Table 2.1) starting on d 1 and intakes were determined daily. Weaning was initiated when calves were consuming at least 0.9 kg/d of calf starter (as-fed basis) for 3 consecutive d. Calves remained in hutches for 7 d following weaning to monitor starter intake prior to moving to group housing. Grass hay (Table 2.1) was provided free-choice in addition to calf starter when calves were pair-housed until transport to FPAC and total DM intakes were determined weekly. At approximately 11 wk of age, calves were transported from PDREC to FPAC and individually housed as outlined above. Transition diets consisted of equal proportions (as-fed basis) of texturized calf starter and assigned post-weaning grain mix fed at 75% of the total diet with the remainder as chopped hay for 2 d following arrival, and then adjusted to 75% treatment grain mix and 25% calf starter for 2 d. Calves were fed transition diets for 4 d then full treatment diets for 3 d prior to initial post-weaning period measurements and diets were top-dressed with 8 g/d of chlortetracycline

(Aureomycin 10G Crumbles; Zoetis) for 5 d during the acclimation to new facilities. Post-weaning treatment diets were low non-fiber carbohydrate (LNFC) or high NFC (HNFC) concentrate mixes with the remainder of the diet offered as chopped hay. Forage:concentrate ratios (DM basis) were 25:75 from 12 to 16 wk, 40:60 from 16 to 24 wk, and 55:45 from 24 to 28 wk of age. Feed was delivered with the concentrate mix top-dressing hay and was offered once per d at 0800 h throughout the study. Ingredient and nutrient composition of concentrate mixes and hay used in the post-weaning period are presented in Table 2.2. Diets were formulated to be isonitrogenous according to NRC (2001) recommendations to allow 0.9 kg/d of ADG for growing Holstein heifers. Feed was initially offered at approximately 2.8% of BW and was adjusted daily to allow for *ad libitum* intake and minimize refusals (≤5% daily). Orts were weighed and sub-sampled daily and composited by treatment each week and frozen at -20°C for later DM and nutrient analysis. Feed ingredients and orts were dried at 60°C in a forced air oven, ground through a 1.0 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ), composited by month, and analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Labs, Ithaca, NY).

2.3.3 Data Collection and Analysis

During the pre-weaning period, calves were weighed and skeletal growth measurements, including hip height (HH), heart girth circumference (HGC), and hip width (HW) were assessed at birth and every 2 wk from assignment to treatment. As weights were taken on prescribed days relative to treatment assignment, weaning weights (WW) were estimated by using ADG for the time period immediately preceding d of

weaning unless the calf was weaned on a scheduled weigh date. Health scores were assessed daily during the pre-weaning period according to the following scale (Heinrichs et al., 2003): for fecal scoring, score 1 = normal, 2 = soft to loose consistency with abnormal coloring and odor, 3 = loose to watery consistency with strong odor, 4 = watery consistency with strong odor, mucus, and slight blood, and 5 = clear, watery consistency with mucus and/or blood; for respiratory scoring, score 1 = normal, 2 = slight cough, 3 = moderate cough, 4 = moderate to severe cough, and 5 = severe and chronic cough; and for general appearance scoring, score 1 = normal and alert, 2 = ears drooped, 3 = head and ears drooped, dull eyes, and slightly lethargic, 4 = head and ears drooped, dull eyes, and lethargic, and 5 = severely lethargic. A scour day was considered if the fecal score was > 3. Blood samples (10 mL/tube) were collected via jugular venipuncture into evacuated blood tubes containing no anticoagulant or lithium heparin (BD Diagnostics, Franklin Lakes, NJ) at birth and every 2 wk (heparinized tubes only). Serum and plasma were aspirated following centrifugation (2500 x *g* for 15 min at 4°C) and frozen at -20°C for later analysis. Serum total protein was determined on samples taken between 1 and 3 d of age (procedure no. 0250; Stanbio Laboratory Inc., San Antonio, TX) to determine passive transfer of immunity status, and plasma was analyzed for plasma urea N (PUN; procedure no. 0580; Stanbio Laboratory Inc.) and glucose (procedure no. 1070; Stanbio Laboratory Inc.) on samples taken at birth to 8 wk of age. During the post-weaning period, BW were taken every 2 wk starting at 12 wk of age, and skeletal measurements described above with the addition of withers height (WH) and body condition score (BCS) was assessed monthly on a scale of 1 to 5 (1 = emaciated, 5 = obese; Edmonson et al., 1989) by 2 evaluators and averaged. Blood was collected approximately 4 h after

feeding monthly and analyzed for PUN and glucose as described above. Rumen fluid was obtained following blood collection and as described by Dennis et al. (2012) at 12, 16, 20, 24, and 28 wk of age using an esophageal tube analyzed for pH, VFA, and rumen NH3. Rumen fluid pH was immediately determined (model EL2; Mettler-Toledo, Columbus, OH), and two 10 mL samples of fluid were acidified using 25% w/v meta-phosphoric acid (4:1 sample:acid ratio) and frozen at -20°C for later analysis. Rumen fluid samples were analyzed for VFA and NH3 as outlined by Fraley et al. (2015).

2.3.4 Calf Harvest and Rumen Tissue Collection

Male calves enrolled in this study were harvested at 12 (n = 6) or 28 wk (n = 12) of age to determine pre- and post-weaning nutrition effects on rumen development. For harvest measurements, 3 calves/treatment were utilized per recommendations outlined by Lesmeister et al. (2004) to detect differences in papillae length and width as indicators of rumen development. All calves remained on full-feed prior to harvest in order to obtain a representative digesta sample from the reticulorumen following euthanasia. Calves that were 12 wk old at harvest were fed calf starter allowance at 0600 h and harvested starting at approximately 0900 h. Calves that were 28 wk old at harvest were transported to PDREC from FPAC within 24 h prior to harvest and remained on treatment diets while at PDREC. Calves shipped from FPAC were harvested the following morning starting at approximately 0900 h. Calves were processed at the Purdue Meat Science Laboratory on main campus and euthanasia was carried out using penetrative captive-bolt stunning and exsanguination. Prior to euthanasia, calves were provided unrestricted access to water and live weights were taken. The total gastrointestinal tract was removed within 15 min

of stunning and the reticulorumen and abomasum were ligated at the cardiac sphincter and pyloric sphincter, respectively, removed from the remaining tract and mesenteric adipose tissue, and weighed full. The omasum and abomasum were subsequently removed and weighed full, and the reticulorumen was weighed full. The exterior of the reticulorumen was washed prior to digesta removal. An incision was made dorsally from the reticulum to the caudal dorsal sac. Digesta was sub-sampled from 4 regions [cranial dorsal (CrD), cranial ventral (CrV), caudal dorsal (CaD), and caudal ventral (CaV)], composited by calf, and the sub-sample was split for use in DM determination and rumen fluid extraction. Digesta for DM determination was immediately bagged and placed on ice, while digesta for rumen fluid extraction was immediately squeezed through 4 layers of cheesecloth and pH was determined as described earlier. Two 10 mL samples of rumen fluid were acidified as described earlier and frozen at -20°C for later analysis. The reticulorumen was washed with water to remove all remaining digesta, re-weighed, and opened to expose the main regions of the organ according to Lesmeister et al. (2004). Two tissue samples (approximately 3 cm × 10 cm each) were cut from each region, with 1 sample placed in 0.9% saline and stored at 4°C for wet tissue dissection and 1 sample stapled to a wooden tongue depressor and fixed in 10% neutral-buffered formalin (10:1 formalin:tissue volume ratio) for histology. Wet tissue dissection was performed within 24 h of sample collection and entailed sectioning rumen tissue samples into 2 sub-samples (approximately 2.5 cm × 2.5 cm), weighing wet tissue, removing epithelial and mucosal tissue from smooth muscle, reweighing separated tissue, and drying at 100°C for 24 h to determine DM proportions of tissue sections. Preserved rumen tissue was allowed to fix for 72 h then subsequently placed in 70% ethanol until embedded in

paraffin blocks for histology or sub-sampled for hand measurements. Tissue blocks and histology slides (hematoxylin and eosin staining) were prepared by the Purdue Histology and Phenotyping Laboratory in the College of Veterinary Medicine. Slides were prepared for each region described above (1 to 5 slides/region per calf) by mounting 5 μm sections onto positively charged slides. Papillae length and width were measured using prepared tissue slides as described by Hill et al. (2005) with the following modifications. For each calf, 3 to 6 papillae were identified and photographed using a digital microscope (BX40F-3; Olympus Optical Co., Ltd., Center Valley, PA) and stereology software (Stereo Investigator 10; MBF Bioscience, Williston, VT). Length and width were measured in a photograph editor (Photoshop CC 2014; Adobe Systems Inc., San Jose, CA) by tracing straight lines across the length and width of each papilla from digital images. Length was determined starting at the base and extending to the tip of each papilla. Width was determined by tracing 3 to 6 lines perpendicular to the length and averaging the values. Hand measurements of tissue morphology were performed by one evaluator using 1 cm^2 punch biopsies of 70% ethanol preserved tissue. All papillae were counted on each punch biopsy (2 per calf from CrV and CaV regions) then 6 to 10 representative papillae were excised and measured using 1 mm^2 graph paper. Priority was given to ventral region samples as papillae length was too short in dorsal regions to excise and accurately hand measure using methods described by Puch et al. (2012).

2.3.5 Statistical Analysis

Data were analyzed by period and overall (birth to 28 wk of age) to determine treatment effects as well as potential interactions of pre- and post-weaning nutrition.

Calves were assigned to treatments at birth in a randomized complete block design with 2×2 factorial arrangement of treatments and blocked by birth date. One heifer died during the pre-weaning period due to causes unrelated to treatment (HI+LNFC). Growth and intake data were analyzed as repeated measures (Littell et al., 1998), as well over each period (birth to weaning, birth to 11 wk of age, and weaning to 11 wk of age for pre-weaning; 12 to 28 wk for post-weaning; and birth to 12 to 28 wk for overall interactions) using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with calf as the experimental unit. Treatment, time, and the interaction of the two variables were included in statistical models as fixed effects and starting measurements were included as covariates where appropriate. Calf nested within block was considered random for repeated measures analysis (growth, intake, blood metabolites, and rumen fermentation parameters) and block was considered random for overall analysis during each period. For harvest measurements, age at harvest was included as a covariate to adjust for age differences within block and foregut and reticulorumen weights were analyzed as-excised and relative to live and hot carcass weights to account for potential differences in gut-fill. Variance-covariance matrix structures were evaluated for each repeated measures model using simple, first order auto-regressive, compound symmetry, and unstructured covariance structures and were selected for each model based on the lowest Bayesian information criterion fit statistic. Least squares means and standard errors of the mean are reported on a per calf basis and mean differences were separated using the Tukey-Kramer method. When interactions of fixed effects were significant, the SLICE option was used to determine the treatment significance at the various time points. Statistical differences were considered significant at $P \leq 0.05$ and trends at $0.10 \geq P > 0.05$.

2.4 Results and Discussion

2.4.1 Pre-weaning Growth Performance, Intakes, and Feed Efficiency

Weight and skeletal growth responses to pre-weaning nutrition are presented in Tables 2.3 and 2.4. Calves fed HI were 3.9 kg, 6.2 kg, and 6.1 kg heavier at 2, 4, and 8 wk of age, respectively, compared to CONV ($P < 0.01$). Additionally, calves fed HI were 18.0 kg heavier at weaning than calves fed CONV ($P < 0.01$); however, as calves were weaned according to calf starter intake, weaning age averaged 65 d for calves fed HI compared to 50 d for calves fed CONV ($P < 0.01$). Advantages in BW for calves fed HI can be attributed to a 14.7% increase in ADG over CONV from birth to 8 wk of age (0.78 vs. 0.68 kg/d; $P < 0.01$). Daily gain from birth to weaning and weaning to 11 wk of age was also improved 18.2 and 20.7%, respectively, for calves fed HI compared to CONV ($P < 0.05$). However, as calves were weaned 15 d sooner when fed CONV, a tendency for calves previously fed CONV to have greater ADG from 8 to 11 wk of age was observed (Figure 2.1) and may be attributed to a compensatory response in DM intake for CONV following removal of MR from the diet (discussed below). Similar responses in gain to those in this study were observed by Cowles et al. (2006), Raeth-Knight et al. (2009), and Hill et al. (2010) for calves fed MR with similar nutrient profiles and feeding rates to those used in this study. Cowles et al. (2006) reported a 13.8% increase in ADG for calves receiving a 28% CP, 20% fat MR compared to a 20% CP, 20% fat MR weaned at 8 wk of age. The authors, in contrast to our findings, observed a 0.53 kg/d reduction in ADG for calves fed a higher CP MR during the week of weaning compared to calves fed a lower CP MR (Cowles et al., 2006). This was attributed to low

calf starter intakes pre-weaning (< 0.9 kg/d) for calves fed higher CP MR compared to lower CP MR (Cowles et al., 2006). Raeth-Knight et al. (2009) reported improved ADG for calves fed a 28% CP, 16% fat MR at 12.5% and 16.7% DM feeding rate compared to a 20% CP, 20% fat MR at 13.9% DM feeding rate, with significant advantages in BW apparent beginning at 2 wk of age. Hill et al. (2010) observed improved pre-weaning ADG for calves fed a 28% CP, 20% fat MR fed at 1.09 kg of DM/d compared to a 20% CP, 20% fat MR fed at 0.44 kg DM/d, but similar ADG in the immediate post-weaning period between treatments. Taken together, these results support that feeding MR with enhanced nutrient profiles pre-weaning increases weight gains; however, advantages in growth rates can be diminished in the immediate post-weaning period, particularly if calf starter intake is not adequate to maintain pre-weaning ADG. Terre et al. (2007) and Hill et al. (2010) suggested that calves fed enhanced MR programs experience lags in growth rate immediately post-weaning due to reduced calf starter digestibility. As calves in the current study were weaned based on starter intake, it is possible that calves fed CONV utilized nutrients from calf starter more efficiently than calves fed HI immediately following weaning as ADG was 16.9% greater from 8 to 11 wk of age compared to 6 to 8 wk of age for calves fed CONV ($P < 0.01$), whereas ADG was similar during the same periods for calves fed HI (Figure 2.1). As calves fed CONV had more accumulated calf starter intake before 11 wk of age compared to calves fed HI, longer and earlier exposure to greater fermentation acid concentrations may affect the ability of the calf to utilize dry feed. When fed higher volumes of MR, appreciable amounts of calf starter intake are delayed, resulting in less total exposure to rumen fermentation acids before 11 wk of age.

Skeletal growth exhibited similar responses to pre-weaning nutrition as BW and ADG (Table 2.4). Hip heights, HW, and HGC were improved 2.7%, 3.6%, and 3.7%, respectively, for HI compared to C at 8 wk of age; however, at 12 wk of age, HH were similar between HI and CONV ($P = 0.24$), and HGC only tended to be greater for calves fed HI pre-weaning ($P = 0.08$). Total gain in HH, HW, and HGC were greater for calves fed HI from birth to 8 wk of age. However, from 8 to 12 wk of age, calves previously fed CONV gained 37% more HH (6.3 vs. 4.6 cm; $P < 0.01$) and 30% more HGC (9.1 vs. 7.0 cm; $P = 0.01$) than calves previously fed HI. Previous studies have reported similar skeletal growth responses when higher CP MR was compared to lower CP MR (Brown et al., 2005b; Cowles et al., 2006; Davis Rincker et al., 2011). In contrast, Blome et al. (2003) and Bartlett et al. (2006) did not observe differences in withers height for calves fed MR with increasing CP content (14% to 26% CP), though HGC and body length were greater for calves fed higher CP MR in both studies. However, feeding rates ranged from 1.25% to 1.75% of BW on a DM basis and calf starter was not provided during the previous studies, which may partially explain skeletal growth as calves were consuming less than 2.0% of BW on a DM basis daily. In general, our data support previous research reporting increased frame growth and weight gain pre-weaning when higher CP MR is provided. This is mostly due to increased nutrient intakes for calves fed HI, particularly increased CP intake which likely resulted in increased lean tissue growth.

Starter, MR, and total DM intakes to weaning were analyzed as a single time point and intakes over time were analyzed as repeated measures and reported for weekly and biweekly periods (Table 2.5 and Figures 2.2 to 2.4). However, as calves were weaned based on intake and calves were weaned at intermediate times relative to other

measurements, repeated measures analysis of intake is confounded with removal of MR from the diet and responses should be interpreted with this in mind. Total starter intake to weaning was similar between treatments, averaging 25.6 and 27.3 kg for CONV and HI, respectively. Total MR intake to weaning was 100.9% greater for calves fed HI ($P < 0.01$), given increased total solids allowed per d as designed and the longer milk feeding period. Total DM intake to weaning, therefore, was 58.9% greater for calves fed HI compared to CONV ($P < 0.01$). Additionally, ME and CP intakes were 70.0% and 88.2% greater, respectively, for calves fed HI compared to CONV from birth to weaning. Following weaning to 11 wk of age, total DM intake (starter + hay) was 55.9% greater for calves previously fed CONV compared to HI ($P < 0.01$); this resulted in similar total DM intake from birth to 11 wk of age between treatments (165.2 and 161.6 kg for CONV and HI, respectively). When analyzed as repeated measures, a treatment×time interaction was observed for all intake measurements. Calves fed HI consumed significantly more DM per d compared to CONV from birth to 2 wk ($P < 0.01$) and 2 to 4 wk of age ($P < 0.01$); yet, total DM intakes were similar from 4 to 6 wk of age. After 6 wk of age, total DM intake was greatest for calves fed CONV ($P < 0.01$; Figure 2.2), mostly driven by greater solid feed intake after weaning for calves fed CONV (Figure 2.3). When expressed as % of BW, total DM intake was greatest for calves fed HI at 2 wk of age ($P < 0.01$); however, intakes were similar between treatments at 4 wk of age and increased for calves fed CONV at 6 wk of age and thereafter ($P < 0.01$; Figure 2.4). Terré et al. (2007) and de Passille et al. (2011) reported similar responses in starter intake to those observed in the current study, as calves fed low volumes of MR (Terre et al., 2007) or pasteurized milk (de Passille et al., 2011) consumed more starter than calves fed high volumes of

milk prior to weaning at a common age. This likely plays a crucial role in rumen tissue development as calves fed CONV have more potential for solid feed digestion and rumen tissue exposure to VFA necessary to develop the rumen epithelium.

Feed efficiency (total gain:total DM intake; G:F) from birth to weaning (adjusted for weaning age), weaning to 11 wk of age (adjusted for sex), and birth to 11 wk of age (adjusted for weaning age and sex) were similar between treatments (Table 2.5). Contrary to the current study, several experiments have reported greater feed efficiency for Jersey (Bascom et al., 2007) and Holstein (Cowles et al., 2006) calves fed higher planes of nutrition pre-weaning, attributed to more digestible nutrients being supplied in the liquid diet. However, other studies have reported results similar to the current study for feed efficiency (Hill et al., 2007b; Hill et al., 2010; Stamey et al., 2012; Bach et al., 2013). Hill et al. (2010) reported feed efficiency values from birth to 8 wk of age slightly lower than those observed in the current study for calves fed 0.44 kg DM of a 21% CP, 21% fat MR or 0.66 kg DM of a 27% CP, 17% fat MR. As total solids offered in the current study were 240 to 480 g more per d than those offered by Hill et al. (2010), greater feed efficiency was likely a result of greater nutrient delivery. Additionally, DM and OM digestibility of calf starter has been shown to increase following weaning for calves fed lower amounts of MR compared to those fed higher volumes of MR (Terre et al., 2007) or greater than 0.66 kg DM of MR powder/d (Hill et al., 2010), which may partially explain similar feed efficiency observed in the current study. Bartlett et al. (2006) compared increasing levels of CP in isocaloric MR formulations (14 to 26% CP, 20% fat) fed at 1.25 or 1.75% of BW and observed a quadratic response in feed efficiency with efficiency maximized at 22% CP in MR and not numerically different

from 26% CP. However, calves fed 26% CP MR at 1.75% of BW feeding rates exhibited the greatest lean tissue BW gain overall compared to calves fed at 1.25% of BW (Bartlett et al., 2006). As calf starter was not provided in the previously described study, discrepancies in feed efficiency values compared to the current study may be attributed to the provision of solid feed before weaning. As DM from MR was restricted to less than 1.75% of birth BW for calves fed CONV, calves fed CONV likely increased calf starter intake in response to a deficit in nutrients provided by MR feeding. Interestingly, male calves were significantly less feed efficient than female calves from birth to 11 wk of age (0.374 vs. 0.460; $P = 0.01$), which could be attributed to 44.2% greater post-weaning DM intake for male calves ($P < 0.01$), as ADG and G:F were not affected by sex pre-weaning. Sweeney et al. (2010) observed similar sex responses for calf starter intake following weaning, as male calves consumed approximately 36% more starter than female calves from 6 to 7 wk of age.

2.4.2 Pre-weaning Blood Metabolites

Blood metabolites analyzed in the pre-weaning period are reported in Figures 2.5 and 2.6. Plasma glucose concentrations were significantly elevated for calves fed HI ($P < 0.01$; Figure 2.5). Increased glucose was likely a result of increased total intake of ME and lactose from MR for HI-fed compared to CONV-fed calves. Glucose significantly decreased over time ($P < 0.01$) regardless of treatment, most prominently from birth to 2 wk of age and 6 to 8 wk of age. Declining blood glucose concentrations with age have also been observed in MR-fed (Wijayasinghe et al., 1984) and whole milk-fed (Quigley et al., 1991) calves as a result of reduced usage of glucose as a predominant energy

source and increased reliance on propionate and butyrate from rumen fermentation of solid feeds. Under the conditions of the current study, it is difficult to separate effects of calf starter consumption and MR intake when discerning glucose responses as calves had access to calf starter from the start of the study. Quigley et al. (2006) observed reductions in blood glucose concentrations for calves fed a conventional MR program and weaned at 4 wk of age compared to calves fed a higher CP MR program and weaned at 8 wk of age. Reduced circulating glucose was likely in response to changes in nutrient source, as calf starter intake was 22% higher for calves fed a conventional MR program compared to higher CP MR program (Quigley et al., 2006). Average weaning age was 65 d for calves fed HI, which would suggest that elevated glucose for calves fed HI through 8 wk of age was attributed mostly to MR consumption, whereas a larger proportion of the circulating glucose concentrations for calves fed CONV was likely attributed to calf starter consumption from 6 to 8 wk of age.

Concentrations of PUN were similar between treatments from birth to 8 wk of age; however, a treatment×time interaction was observed (Figure 2.6) as PUN was significantly greater at 8 wk of age for calves fed CONV ($P = 0.01$). Cowles et al. (2006) observed similar increases in blood urea N associated with increased starter consumption prior to weaning for calves receiving a 20% CP, 20% fat MR fed at 562 g of MR/d compared to 28% CP, 20% fat MR fed to meet intakes of 0.27 Mcal/kg of $BW^{0.75}$. As calves in the current study were weaned based on calf starter intake, it stands to reason that corresponding increases in PUN observed for calves fed CONV may be related to increased fermentation of solid feeds in the rumen, as total DM intake was similar but calf starter intake was significantly higher for calves fed CONV at 8 wk of

age. Total CP intake was greater for calves fed HI at 8 wk of age ($P < 0.01$); however, CP intake from starter was significantly greater at 8 wk of age for calves fed CONV greater compared to calves fed HI ($P < 0.01$), which may partially explain the increase in PUN observed in the current study.

2.4.3 Rumen Fermentation Parameters Immediately Post-weaning

Prior to transporting calves to FPAC, rumen fluid samples were collected to determine potential differences in rumen fermentation associated with MR feeding program. Rumen fermentation profiles for calves at 11 wk of age are outlined in Table 2.6. Rumen pH and rumen NH3 concentrations were similar between MR treatments, averaging 5.6 and 10.3 mg/dL NH3, respectively. However, total VFA concentrations tended to be 14.6 mM greater for calves fed CONV compared to HI ($P = 0.06$). Greater VFA concentrations for calves fed CONV may be associated with greater total DM intake observed following weaning or less dilution of VFA in the rumen. Substrate availability in *in vitro* fermentation studies is often a limiting factor to VFA production (Dijkstra, 1994) and it is logical that increased feed intake would result in an increase in total VFA. Although total VFA concentrations were increased for calves previously fed CONV, VFA profiles were similar between treatments. As the diet immediately post-weaning was similar, no differences in VFA profiles were expected. Fermentation profiles observed in the current study were similar to those reported in calves fed texturized calf starters with (Coverdale et al., 2004) and without hay provision (Lesmeister and Heinrichs, 2004). Higher starch calf starters often result in rumen pH

below 6.0, which favor fermentation profiles with lower acetate:propionate ratios (Coverdale et al., 2004) similar to those reported in this study.

2.4.4 Pre-weaning Health Measurements

Health measurements for calves from birth to 7 d post-weaning are reported in Table 2.7. Serum total protein was analyzed to determine initial status of passive immunity transfer, and only 1 male calf fell below a threshold of 5.0 g/dL of serum total protein (Donovan et al., 1998). Average scour scores were significantly higher for calves fed HI ($P = 0.03$). When analyzed by 2 wk periods, calves fed HI had significantly higher scour scores from birth to 2 wk (9.3%; $P < 0.01$), 2 to 4 wk (26.6%; $P < 0.01$), and 4 to 6 wk of age (15.9%; $P < 0.01$), but not from 6 to 8 wk of age ($P = 0.62$) compared to CONV. Calves fed HI had, on average, 3 more scour days (score ≥ 3) from birth to 1 wk post-weaning ($P < 0.01$). Other studies have also reported higher fecal scores and increased number of scour days when pre-weaned calves were fed higher planes of nutrition (Quigley et al., 2006; Raeth-Knight et al., 2009; Davis Rincker et al., 2011), particularly within the first 2 wk of life (Diaz et al., 2001). When variable amounts of a 28% CP, 17% fat MR were fed to Holstein bull calves (454 to 908 g/d of DM), growth was improved compared to feeding a conventional MR program, but more calves became sick resulting in more scour days and increased veterinary treatment costs (Quigley et al., 2006). Granted, 63% of calves exhibited failure of passive transfer (plasma IgG concentrations < 10 g/L), shipping stress was induced, and calves were challenged with coronavirus in the previous study (Quigley et al., 2006); however, these results indicate there is a potential negative effect when feeding high planes of nutrition to stressed calves

or when pathogen loads are high. Increased fecal fluidity associated with higher DM inclusion in pasteurized milk (Glosson et al., 2015) and MR (Jenny et al., 1982) is likely due to differences in osmolality between liquid feed and the small intestine, thereby altering water absorption in the hindgut and increasing the chance for dehydration. Increased dehydration associated with increased fecal fluidity could result in increased administration of electrolytes, number of veterinary treatments, and an overall increase in total costs during the pre-weaning period. However, greater fecal scores do not always correspond to reduced health status (Diaz et al., 2001), as milk and MR programs that increase DM consumption from liquid feed can increase passage rate, resulting in looser stools. In the current study, it does not appear that increased fecal scores and scour days translated to reduced health status in calves fed HI, as the absolute difference in fecal scores between MR treatments were not biologically different. Respiratory and general appearance scores were similar between treatments overall and for 2 wk periods up to 8 wk of age. After 8 wk of age, 13 instances of scour days (CONV = 10, HI = 3), no respiratory scores > 1, and no general appearance scores > 1 were observed. Scour day observations for calves fed CONV after 8 wk were predominately loose and foamy, suggesting potential bouts of lactic acidosis or excessive hindgut fermentation associated with high starter consumption.

2.4.5 Post-weaning Growth Performance, Intakes, and Feed Efficiency

Weight and skeletal growth responses to grower diets are presented in Tables 2.8 and 2.9. A treatment×time interaction was observed for BW, as no differences in BW were detected at 16 and 20 wk of age, but was significantly greater for calves fed HNFC

compared to LNFC at 24 and 28 wk of age ($P \leq 0.01$). Calves fed HNFC were also 9.7 kg heavier on average than calves fed LNFC at 28 wk of age ($P < 0.01$). Furthermore, overall ADG was 8.7% greater for HNFC compared to LNFC ($P = 0.04$). Average daily gain increased over time ($P = 0.01$) regardless of treatment, most likely due to the increase in DM intake over time (discussed below). Pirlo et al. (1997) evaluated diets for prepubertal Italian Friesian heifers ranging from 90 to 110% of requirements for TDN and CP, respectively, and reported increased ADG from 100 to 200 kg of BW when TDN and CP were 110% of animal requirements, though only significant effects of dietary TDN were observed. The authors also observed that decreasing TDN and increasing CP did not result in acceptable growth rates (Pirlo et al., 1997), illustrating the importance of satisfying energy requirements to achieve targeted ADG in weaned heifers. As diets in the current study were formulated to be isonitrogenous and vary in ME by altering NFC and NDF concentrations, differences in ADG were likely attributed to energy availability from different carbohydrate sources. However, growth rates in the current study were greater than those predicted by the NRC (2001) model and would be considered acceptable for prepubertal dairy calves at this age. Frame measurements were inconsistently affected by post-weaning diet, as tendencies for treatment×time interactions were observed for HH and HW ($P = 0.08$), but not WH or HGC. Calves fed HNFC tended to be taller at HH ($P = 0.06$) and wider at HW ($P = 0.08$) than calves fed LNFC at 28 wk of age. A sex effect was observed for HH, WH, HW, and HGC, as steers, on average, were significantly taller at the hip (112.5 vs. 111.1 cm; $P < 0.01$) and withers (108.6 vs. 106.9 cm; $P < 0.01$) and exhibited larger HGC (129.4 vs. 127.8 cm; $P = 0.02$), whereas heifers were wider at the hips (30.8 vs. 30.3 cm; $P = 0.05$). Total gain

in HH ($P < 0.01$) and WH ($P = 0.02$) were 19.9 and 14.4% greater for steers compared to heifers from 12 to 28 wk of age. Gabler and Heinrichs (2003a) reported similar skeletal measurements for Holstein heifers weighing 125 to 234 kg fed increasing proportions of CP:ME. The authors did not report a treatment response in growth; however, heifers were limit-fed diets to achieve a similar ADG, which likely explains a lack of treatment response in skeletal growth in their study. In the current study, limited response in skeletal growth, with the exception of HH and HW, may be due to more nutrients being partitioned to BW gain in lieu of frame at this age.

Total DMI (kg/d) was similar between treatments overall, but when expressed as a percent of BW, calves fed LNFC consumed 6.9% more DM as a percent of BW than calves fed HNFC (Table 2.9). Most of the response in DMI was observed from 16 to 20 wk of age ($P \leq 0.10$), but not from 24 to 28 wk of age (Figure 2.7). Energy intake was similar between treatments overall, but a treatment×time effect was observed for ME (Figure 2.8). As DMI increased for calves fed LNFC, energy intakes were similar from 12 to 24 wk of age; however, because DMI was similar from 24 to 28 wk, ME intake was 1.43 Mcal/d greater for calves fed HNFC at 28 wk of age ($P = 0.03$) due to increased energy density of the diet. These results illustrate that at younger ages with higher energy demands for growth, calves will increase DMI to meet energy requirements regardless of diet composition. Mertens (1994) reported that as energy concentrations in diets increase, animals will respond by reducing DMI. Additionally, increased dietary NDF typically results in decreased DMI related to increased gut fill (Allen, 2000), yet calves in the current study fed LNFC did not appear to exhibit physical intake restriction until 24 wk of age when the dietary forage level was increased. Increased passage rate may

partially explain increased intake observed earlier in the study (16 to 20 wk of age), which in turn would reduce diet digestibility and result in reduced performance. Higher inclusion of non-forage fiber sources, such as cottonseed hulls, soybean hulls, and wheat bran, has been shown to increase intake and passage rate in lactating dairy cow diets (Firkins, 1997; Grant, 1997). This is particularly true when there is less potential to trap smaller particles in the rumen fiber mat (Grant, 1997), which would occur when forage is included in diets at lower F:C ratios. Effects of fiber source may also explain some of the DMI responses observed, particularly later in the study when hay occupied a larger proportion of the diet. Overall, CP intake (kg/d) was also similar between treatments as expected, as diets were formulated to provide the same CP content assuming similar intakes. However, a treatment×time effect was observed (Figure 2.9) with calves fed LNFC consuming 15.3% more CP per d than calves fed HNFC from 12 to 16 wk of age ($P = 0.02$). Intake of CP as a percent of BW also exhibited a treatment×time effect in favor of increased CP intake for LNFC from 12 to 16 wk of age ($P < 0.01$) and 16 to 24 wk of age ($P = 0.05$). Despite increased CP intake and similar energy intake from 12 to 16 wk of age, feeding LNFC diets resulted in lower ADG throughout the post-weaning period. This may be due to differences in carbohydrate availability in the rumen relative to N availability, as PUN and rumen NH3 were affected by diet (discussed below). Reduced performance despite increased CP intake suggests inability of calves fed LNFC to utilize CP efficiently early in the study, perhaps indicating reduced capacity for ruminal digestion of high fiber feeds at this age.

Total NDF intake was 22.7% greater for LNFC ($P < 0.01$) and starch intake was 54.0% greater for HNFC ($P < 0.01$) overall as anticipated. Total NDF intake as a percent

of BW was also significantly increased for calves fed LNFC compared to HNFC (1.7% vs. 1.2%, respectively; $P < 0.01$). Forage NDF intake was similar between treatments overall ($P = 0.85$), but a treatment×time interaction was observed ($P < 0.01$) with calves fed LNFC consuming 12.6% more fNDF from 12 wk to 16 wk, but 3.6 % less fNDF from 24 wk to 28 wk. As a percent of BW, however, fNDF intake was similar between treatments and over time. These responses in NDF and fNDF intake closely follow total DMI, as calves fed LNFC had greater DMI early in the post-weaning period but converged to similar intakes when hay inclusion was increased in the diet. Differences in carbohydrate intake were designed to differ among treatments and differences in intake were expected; however, as the LNFC diet was significantly greater in total NDF content, we expected intakes to be reduced for calves fed that diet. Greater inclusion of soybean hulls, cottonseed hulls, and wheat bran in the LNFC grain mix may have increased passage rate in the current study for calves fed LNFC, resulting in increased DMI as a percent of BW from 12 to 24 wk. Grant (1997) indicated that lower forage diets have less potential for entrapment of small particles, resulting in greater passage rate of non-forage fiber sources and less rumen retention and digestion. However, energy availability in non-forage fiber sources would be less compared to starch and increased DMI could have been driven by the need to satisfy energy requirements for calves fed LNFC which would theoretically increase passage rate. Diets including cottonseed hulls at 7.8% of dietary DM for early lactation cows increased DMI approximately 8% over diets without cottonseed hulls (Kononoff and Heinrichs, 2003). Similarly, milk-fed Holstein calves exhibited greater starter intakes when cottonseed hulls were included in concentrations up to 15% of the starter DM (Hill et al., 2009a). Inclusion of other non-forage fiber sources

has also been shown to increase DMI and passage rate in lactating dairy cattle (Firkins, 1997; Grant, 1997), which may partially explain DM and NDF intake responses seen in the current study. As DM and fNDF intakes were similar but total NDF intakes differed between treatments from 24 to 28 wk, intake regulation for growing calves may switch from predominately chemical to physical regulation as forage NDF inclusion, not total NDF, increases in the diet. Hoffman et al. (2008) reported DM and NDF intakes that were 18.9% and 50.3% lower, respectively, for pen-fed Holstein heifers fed diets with similar CP and NDF content at a similar BW to heifers in the current study. The authors did not report diet composition for pen-fed heifers, and reasons for disagreement in DM and NDF intakes between Hoffman et al. (2008) and the current study are unclear. However, it is common to feed growing dairy heifers diets containing large proportions of dry and ensiled forages, which have the potential to limit intake due to effective fiber content. As NE_m for diets during the post-weaning period were 16.8% lower, on average, than those reported by Hoffman et al. (2008), increased intake responses may indicate heifers were consuming more feed to meet maintenance energy requirements. Casper et al. (1994) reported DM intakes for 3 mo-old Holstein heifers of 3.31% of BW, similar to those observed for HNFC-fed calves in the current study.

Feed and nutrient efficiency values are presented in Table 2.9. Feeding HNFC resulted in a 12.7% improvement in G:F compared to feeding LNFC ($P < 0.01$). Interestingly, a tendency for a treatment×time effect was observed ($P = 0.06$), as G:F was improved for calves fed HNFC from 12 to 16 wk ($P < 0.01$) and 16 to 24 wk ($P = 0.01$), but similar from 24 to 28 wk between treatments ($P = 0.48$). Energy efficiency (kg ADG/Mcal ME intake) was also improved for calves fed HNFC ($P = 0.02$), as was CP

efficiency (kg ADG/kg CP intake; $P < 0.01$). The response in feed and nutrient efficiencies is likely a result of increased diet digestibility and fermentability for HNFC compared to LNFC. Geay (1984) reported a curvilinear relationship between the energy retained as protein and ME efficiency for growth, as greater protein deposition is the result of lower ME efficiency since protein accretion is more energetically expensive. In the current study, it appears that despite equal ME intake, carbohydrate source of dietary ME may affect the relationship of protein and energy utilization in growing heifers. This could be partly due to differences in rumen fermentation end products or energy expenditure for digestion, as the net utilization of fiber is energetically less favorable than starch utilization in cattle (VandeHaar and St-Pierre, 2006). Additionally, as ME is a calculated value based on total digestible nutrients in a given feed, differential responses in growth observed when ME intake was equal in the current study suggests energy utilization is dependent on source of ME provided to heifers at this age. This also suggests that calculated energy values in feeds may need to be adjusted for age of the animal being fed, as rumen development in young heifers may partially explain discrepancies in predicting intake and growth by the NRC (2001) model.

2.4.6 Post-weaning Rumen Fermentation Parameters and Blood Metabolites

Rumen VFA, NH3, and pH values are reported in Table 2.10. Total VFA concentrations and rumen pH were similar between treatments throughout the study; however, VFA profiles were altered in response to dietary NFC. Calves fed HNFC had greater proportions of propionate and isoacids (isobutyrate + isovalerate; $P < 0.01$), tended to exhibit greater proportions of butyrate ($P = 0.08$), and lesser proportions of

acetate ($P < 0.01$) compared to calves fed LNFC. Treatment×time effects were observed for molar proportions of acetate ($P = 0.04$) and propionate ($P < 0.01$), as acetate was 3.9% greater for calves fed LNFC from 12 to 16 wk ($P = 0.01$) and propionate was 7.4% greater for calves fed HNFC from 12 to 16 wk ($P = 0.02$). Unexpectedly, calves fed LNFC tended to have lower rumen pH over time than calves fed HNFC ($P = 0.10$), particularly from 24 to 28 wk (6.33 vs. 6.49, respectively; $P = 0.08$). Given that sampling times were 4 to 6 h after the morning feeding, differences in fermentation profiles may be related to fermentation rates of different carbohydrate sources. Additionally, absorption of fermentation acids from the rumen may have been reduced for LNFC-fed calves, as total VFA concentrations from 24 to 28 wk were 13.3% higher for calves fed LNFC ($P = 0.03$). Accumulation of VFA does not usually occur under normal rumen conditions, but may occur when rate of VFA production exceeds absorption or absorption is inhibited (Owens et al., 1998). It is possible that due to the altered rumen fermentation profiles in favor of reduced butyrate as a percent of the total VFA present, rumen epithelial tissue development may have been reduced for calves fed LNFC, resulting in accumulation of VFA. However, increased VFA for LNFC may also have been attributed to greater fiber digestion when hay inclusion was increased from 24 to 28 wk of age.

Rumen NH3 concentrations were significantly greater for calves fed LNFC ($P < 0.01$), despite similar CP intake between treatments. However, RDP made up a larger proportion of dietary CP in LNFC, which may explain increased rumen NH3 observed in the current study. Additionally, the reduction in available carbohydrates may have also decreased N utilization in the rumen. Increased proportions of isoacids observed in

animals fed HNFC, along with reduced concentrations of rumen NH3, likely indicate increased microbial protein production efficiency. Isoacids are primarily produced by catabolism of branched-chain amino acids in the rumen (Andries et al., 1987), suggesting proteolytic activity was enhanced under the HNFC diet. Reductions in rumen NH3 concentrations, in the range of 3 to 8 mg/dL, have been associated with optimal assimilation of nitrogen into microbial CP (Satter and Slyter, 1974) and increased microbial CP efficiency. Conversely, isoacids can also be associated with increased cellulolytic activity in the rumen, as they are required growth factors for cellulolytic bacteria (Andries et al., 1987). Decreased proportions of the isoacids for animals fed LNFC may indicate an increase in celluloytic activity associated with increased dietary NDF and reduced starch, as fiber digesting bacteria would utilize carbon from isoacids to grow.

Blood glucose and PUN concentrations are presented in Table 2.10 and Figures 2.10 and 2.11. Glucose concentrations were elevated for calves fed HNFC compared to LNFC, as starch intake was greatest for HNFC throughout the feeding period. Glucose and insulin concentrations are sensitive to changes in dietary carbohydrates, and have been found to be significantly elevated when sheep and dairy cows are fed low- compared to high-roughage diets (Evans et al., 1975). Similarly, post-prandial serum insulin concentrations were significantly elevated for beef steers fed all-concentrate compared to all-fiber diets, suggesting higher glucose utilization by peripheral tissues in response to greater glucose supply from a high concentrate diet (Schoonmaker et al., 2003). As proportions of propionate were greater for calves fed HNFC, increased circulating glucose is likely related to increased energy utilization from dietary starch.

Increased glucose levels are in agreement with Park et al. (1987) and Abeni et al. (2000) for heifers with greater ADG in response to increased energy intake. Lower propionate production early in the post-weaning period may explain reduced glucose concentrations overall for calves fed LNFC.

Plasma urea N concentrations (Figure 2.11) followed a similar trend as rumen NH3, as PUN was 15.6% higher for calves fed LNFC ($P < 0.01$). Several studies have reported the positive relationship between CP intake and rumen NH3 and blood urea concentrations (Preston et al., 1965; McIntyre, 1970; Slyter et al., 1979), and results in the current study reflect similar responses. Concentrations of PUN ranged from 6.9 to 9.6 mg/dL across all treatments during the study, which has been reported to illustrate optimal N utilization in growing cattle (Byers and Moxon, 1980).

2.4.7 Effects of Pre-weaning Nutrition on Post-weaning Growth and Intake

Post-weaning calf performance in response to pre-weaning plane of nutrition is presented in Figures 2.12 to 2.16. Calf BW analyzed from birth to 28 wk of age exhibited a treatment×time interaction ($P = 0.02$), though BW were only different between treatments during the pre-weaning period and converged at 12 wk of age. Skeletal growth curves from birth to 28 wk of age showed similar trends to BW, though HH were greatest during the pre-weaning period as well as at 16 and 20 wk of age ($P \leq 0.05$) for calves previously fed HI. However, HH were similar between pre-weaning treatments at 28 wk of age. Average daily gain also exhibited a treatment×time effect ($P < 0.01$), as ADG were greater for calves previously fed CONV at 14 and 20 wk of age ($P \leq 0.05$), but were similar at all other time points post-weaning. From birth to 28 wk of

age, ADG was similar between MR treatments ($P = 0.61$). Dry matter intake (kg/d) was similar from 12 to 28 wk of age between MR treatments; however, when expressed as a percent of BW, calves previously fed CONV consumed more DM as a percent of BW from 12 to 16 wk of age compared to calves previously fed HI ($P = 0.05$). This result may be associated with greater intakes observed for calves fed CONV after 56 d of age and may be indicative of increased gut capacity for calves fed CONV early in the post-weaning period.

Data comparing post-weaning responses to pre-weaning nutrition are limited, as few studies report growth measurements beyond 2 wk following weaning. Davis Rincker et al. (2011) compared feeding a conventional MR (21.5% CP, 21.5% fat) to an intensified MR (30.6% CP, 16.1% fat) program and observed similar responses in BW post-weaning to those of the current study. Growth in BW and skeletal size increased for calves fed an intensified MR program through 8 wk of age, but BW were similar from 12 to 100 wk of age while withers height continued to be greater for calves fed the intensified program through 40 wk of age (Davis Rincker et al., 2011). Continued advantages for feeding an increased plane of nutrition pre-weaning with respect to frame height in their study may have been due to greater CP available in the MR as well as the calf starter provided (19.9% CP vs. 24.3% CP for conventional and intensive, respectively), resulting in more CP intake to promote lean tissue and frame growth. Despite similar growth performance between MR programs in the current study from 12 to 28 wk of age for BW and 24 to 28 wk of age for HH, potential management advantages exist when feeding for greater growth rates early. Several studies have illustrated increased prepubertal ADG can reduce age at first conception (Ettema and

Santos, 2004; Davis Rincker et al., 2011) which can result in younger heifers at first calving (Raeth-Knight et al., 2009). This has potential economic implications, as non-productive days are reduced (Davis Rincker et al., 2011); however, cost per kg of gain is typically increased when feeding higher planes of nutrition pre-weaning (Brown et al., 2005b; Raeth-Knight et al., 2009), which may result in similar or potentially higher costs incurred to develop a replacement heifer. Yet, heifers that calve between 22 and 24 mo of age, which often occurs as a result of higher growth rates before puberty, tend to yield more milk in the first lactation (Zanton and Heinrichs, 2005), which could potentially off-set the higher cost of rearing associated with higher MR feeding programs. However, economic comparisons were not made in the current study to evaluate feed costs.

2.4.8 Interaction of Pre- and Post-weaning Nutrition

Effects of the interaction of pre- and post-weaning diets are presented in Table 2.11 and Figures 2.16 to 2.20. Interactions were only observed for growth and intake parameters, as rumen fermentation profiles and blood metabolites were predominately affected by post-weaning treatments. A significant interaction of pre- and post-weaning treatments was observed for BW at 28 wk of age ($P = 0.04$). Calves fed HI pre-weaning with a LNFC post-weaning diet were 9.8 kg and 12.4 kg lighter at 28 wk of age than calves fed CONV or HI pre-weaning with a HNFC post-weaning diet (Figure 2.16). Most of the weight response can be attributed to increased BW gain from 12 to 28 wk of age (Table 2.11), as no interaction was observed for ADG from birth to 28 wk of age. A similar 3-way interaction of pre- and post-weaning nutrition over time was observed for HH (Figure 2.17), as calves fed HNFC, regardless of pre-weaning nutrition, were taller at

the hip compared to calves fed CONV with LNFC ($P < 0.01$). Additionally, calves fed HI with LNFC were tallest at 16 wk of age, but advantage in height declined over time in favor of calves fed HNFC diets, regardless of pre-weaning nutrition. Though an interaction of pre-weaning and post-weaning diets was not observed for DMI, a 3-way interaction of pre- and post-weaning treatment over time was observed ($P < 0.01$; Figure 2.18). Calves previously fed HI with LNFC consumed more DM from 18 to 20 wk of age compared to calves fed CONV with HNFC ($P < 0.01$); however, DMI converged for all treatments from 24 to 28 wk of age. Intake of ME and CP also exhibited a 3-way interaction ($P < 0.01$), following trends seen with total DMI (Figures 2.19 and 2.20). Feed efficiency was greatest for calves fed HI+HNFC and least for calves fed HI+LNFC from 12 to 16 wk of age ($P < 0.01$), which may suggest reduced diet digestibility and rumen function for calves fed high planes of nutrition pre-weaning with low energy, high fiber post-weaning diets. Terré et al. (2007) and Hill et al. (2010) have reported reduced apparent diet DM and fiber digestibility immediately post-weaning for calves fed high planes of nutrition pre-weaning. As ME efficiency was also lowest for calves fed HI+LNFC from 12 to 16 wk, it stands to reason that energy utilization from the LNFC diet for calves previously fed a high plane of nutrition may be linked to the functional capacity of the rumen at this age. Hip height, HGC, and BCS were similar among treatments, thought HW did tend to exhibit an interaction between pre- and post-weaning diets ($P = 0.08$). Similar to BW and ADG, HW were greatest for HI+HNFC and least for HI+LNFC at 28 wk of age. Overall, it appears in order to maintain growth advantages seen with feeding high planes of nutrition pre-weaning, highly digestible, higher energy post-weaning diets should be provided to prepubertal heifers. However, interactive

effects for increased G:F and ME efficiency were segregated to the early post-weaning period (12 to 16 wk of age), suggesting that more data characterizing this period in heifer development is warranted.

2.4.9 Harvest Measurements and Rumen Development

Rumen fermentation parameters for calves harvested at 12 wk and 28 wk of age are presented in Table 2.12. Rumen pH, NH3, and total VFA concentrations were similar between milk feeding treatments for 12 wk-old calves. Unexpectedly, molar proportions of propionate tended to be 11.4% higher for calves fed HI ($P = 0.07$); however, butyrate proportions were 32.2% greater for calves fed CONV ($P < 0.01$). Increased butyrate proportions in rumen fluid for calves fed CONV is likely reflective of earlier weaning ages and more potential for fermentation of solid feeds compared to calves fed HI. Contrary to observations for all calves from 12 to 28 wk of age, rumen NH3 and VFA concentrations were similar between post-weaning treatments at harvest. Rumen pH tended to be lower ($P = 0.08$) and fermentation profiles were altered in favor of greater propionate ($P < 0.01$) and butyrate ($P < 0.01$) proportions for steers fed HNFC, agreeing with values observed with all calves post-weaning. Discrepancies in rumen fermentation characteristics between samples collected during the grower period and at harvest may be related to time relative to feeding. Due to transport protocols used in this study, steers were fed in the morning prior to transport and in the afternoon after arrival at PDREC in two equal meals, which could have influenced rumen fermentation parameters at harvest. Additionally, harvest samples were a single time point when hay inclusion in the diet was

the greatest compared to repeated measurements of rumen fluid which encompassed differing F:C ratios throughout the study.

Harvest measurements, rumen weights, and rumen morphology are reported in Tables 2.13 to 2.16. Live weight at harvest was similar between MR feeding treatments, despite bull calves fed HI having a 12.6 kg numerical advantage compared to bull calves fed CONV at 12 wk of age ($P = 0.17$). Additionally, empty rumen weight as a % of live BW at harvest was also similar between treatments. Hot carcass weight (HCW), dressing %, and other rumen weights did not differ between MR feeding treatments for 12 wk old bull calves. However, calves fed CONV had greater proportions of mucosal tissue to live BW ($P = 0.03$) and HCW ($P = 0.03$) compared to calves fed HI. These results are not completely unexpected given that MR was not removed from the diet until solid feed consumption was adequate to wean calves which resulted in a difference of 15 d at the time of weaning. However, DMI was greater for calves fed CONV post-weaning, which likely would increase rumen mass in response to increased digestion and fermentation of solid feed resulting in greater proportions of absorptive mucosal tissue. Rumen papillae length, width, and surface area were similar between milk feeding treatments for 12 wk-old bull calves. Kristensen et al. (2007) similarly observed no differences in rumen papillae length for calves fed increasing levels of MR from 3.1 to 8.3 kg/d (123 g DM/kg MR), despite increased starter intake for calves fed the lowest MR allowance. As calves in the current study were weaned based on starter intake, adequate solid feed intake likely occurred to initiate rumen fermentation and tissue development prior to 12 wk of age resulting in similar rumen tissue morphology. However, it is unclear if functional differences in VFA and proton transporters on the

rumen epithelium were apparent, as Laarman et al. (2012a) identified that relative abundance of monocarboxylate transporter, isoform 1 mRNA was increased and Na^+/H^+ exchanger, isoform 3 mRNA was decreased in the rumen epithelium of 50 d-old calves fed calf starter compared to calves not fed starter. The authors postulated that the concomitant increase and decrease in expression of each of these genes may increase proton removal from the rumen, indicating an improvement in VFA absorption when calf starter was provided before weaning (Laarman et al., 2012a). While outside the scope of the current study, there may have been functional differences in the absorptive capacity of rumen tissue at 12 wk of age due to MR feeding program despite similar morphology, and future work investigating cellular-level differences in rumen epithelium due to feeding program are warranted.

In response to post-weaning diets, calves fed HNFC tended to be 15.5 kg heavier than those fed LNFC at 28 wk of age ($P = 0.10$). Calves fed HNFC also tended to yield heavier HCW compared to calves fed LNFC ($P = 0.06$). Full and empty reticulorumen weights, ratios of the reticulorumen to BW and HCW, and ratios of mucosa and muscle tissue to BW, HCW, and empty reticulorumen weights were similar with the exception of full reticulorumen:total foregut weight and muscle:HCW. Calves fed HNFC showed a greater proportion of full reticulorumen:total foregut weight ($P = 0.05$), most likely related to numerically greater DM and fNDF intake observed from 24 to 28 wk of age compared to calves fed LNFC. The proportion of rumen muscle tissue to HCW tended to be greater for calves fed LNFC ($P = 0.09$), which would be expected given a diet with greater hay inclusion and total NDF concentration, thereby increasing the potential to stimulate muscular development due to rumination. Interestingly, a pre- by post-weaning

interaction was observed for proportions of muscle:BW and muscle:HCW. Calves fed CONV+LNFC exhibited the greatest proportions of muscle:BW and muscle:HCW compared to calves fed CONV+HNFC ($P < 0.05$), with calves fed HI, regardless of post-weaning treatment, falling intermediate. While most of the response was likely due to post-weaning diet, it is possible that earlier initiation of rumen function due to early starter intake may increase muscle development compared to delayed intake of starter feeds seen with higher MR feeding rates. Coupled with a lesser NFC diet post-weaning, there appears to be an additive effect on muscle tone when compared to BW and HCW.

Hand measurement of rumen papillae are summarized in Tables 2.15 and 2.16. While histological measurements of morphology were similar with the exception of a significant increase in papillae length for 28 wk-old steers fed LNFC (Table 2.13), a pre-weaning × age × region interaction was observed for several morphology measurements when analyzed by hand. At 12 wk of age, papillae length, width, surface area, and papillae density were similar between treatments; however, papillae length, surface area, and surface area ratios were greatest and papillae density was least for calves fed CONV in cranial ventral samples at 28 wk of age compared with caudal ventral samples. This illustrates the need to identify tissue regions when reporting tissue morphology data, as the overall effect of pre-weaning treatment with age was not significant for any measurement. Additionally, this also justifies giving priority to cranial ventral samples when measuring morphology by hand, as differences were detected with a small number of calves per treatment (n = 6 at 28 wk of age). In general, rumen papillae length, surface area, and surface area ratios were greater for 28 wk-old calves compared to 12 wk-old calves, regardless of tissue region. This was expected given increased DMI and substrate

availability for older calves. Surprisingly, no pre- and post-weaning nutrition interactions were observed for tissue morphology, yet pre-weaning treatment affected surface area ($P = 0.02$) and surface area ratios ($P = 0.09$) in cranial ventral, but not caudal ventral tissue samples. Calves fed CONV had greater surface area and surface area ratios compared to calves fed HI at 28 wk of age. This may indicate, similar to muscle:BW proportions, feeding conventional MR programs that encourage early solid feed intake predisposes rumen tissue to exhibit greater surface area, which could allow greater nutrient uptake from the rumen environment. However, increased rumen tissue surface area did not correspond to increased performance of calves fed CONV at 28 wk of age, though BW and frame size were similar to calves fed HI. Developing calf feeding programs that maximize growth from milk or MR while encouraging earlier and greater starter intake before weaning may be prudent for future research objectives.

2.5 Summary and Conclusions

When evaluating conventional compared to high planes of milk replacer nutrition pre-weaning, low compared to high NFC diets post-weaning to 28 wk of age, and the interaction of pre-weaning and post-weaning nutrition, calves fed high planes of nutrition pre-weaning with a high NFC diet post-weaning were heaviest with the highest BCS at 28 wk of age. Additionally, feed and energy efficiency were improved for calves previously fed a high plane of nutrition on a high NFC diet from 12 to 16 wk of age. Feeding a high plane of nutrition pre-weaning resulted in increased growth rates as a result of increased nutrient intakes; however, feed efficiency was similar between milk replacer feeding programs and some advantages in growth rates and skeletal size were

reduced or lost following weaning. Feeding high NFC diets post-weaning resulted in greater growth rates, reduced feed intake, and improved feed, energy, and protein efficiency from 12 to 28 wk of age. Additionally, as forage inclusion increased in the post-weaning period, feed intake became more restricted by gut capacity and forage fiber content than total dietary fiber. Despite advantages in growth rates and manipulation of rumen fermentation in favor of increased proportions of butyrate for calves fed a high NFC diet, rumen tissue morphology was similar between post-weaning diets. Proportions of muscle tissue in the rumen increased when calves were fed a low NFC diet post-weaning, which may play a role in regulating intake and rumen development as calves age and consume more forage and high-fiber feeds. These results suggest calves fed a high plane of nutrition pre-weaning should continue to receive high planes of nutrition post-weaning to maintain growth advantages and rumen development may continue well into the post-weaning period.

2.6 Acknowledgements

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Table 2.1. Nutrient analysis (± s.d.) of conventional milk replacer (CONV), high nutrition plane milk replacer (HI), calf starter, and hay offered to calves from birth to 12 wk of age.

Item[1]	CONV[2]	HI[3]	Starter[4]	Hay[5]
DM	96.3 (0.4)	96.0 (0.7)	89.8 (0.7)	92.4 (0.6)
CP	22.6 (0.4)	28.9 (0.4)	24.4 (1.5)	17.4 (2.3)
Crude fat	21.3 (0.8)	21.2 (1.8)	6.4 (1.2)	2.9 (0.4)
ME[6], Mcal/kg	4.38 (0.04)	4.50 (0.10)	3.41 (0.06)	2.15 (0.05)
NE_m[7], Mcal/kg	3.10 (0.04)	3.19 (0.10)	2.35 (0.06)	1.29 (0.05)
NE_g[8], Mcal/kg	2.18 (0.02)	2.21 (0.07)	1.41 (0.04)	0.55 (0.04)
NFC[9]	nd[10]	nd	49.6 (3.3)	14.5 (3.8)
NDF	nd	nd	12.3 (1.0)	56.3 (4.3)
ADF	nd	nd	7.0 (0.9)	35.0 (2.6)
Ca	0.88 (0.02)	0.89 (0.06)	1.15 (0.10)	0.59 (0.15)
P	0.92 (0.02)	0.88 (0.05)	0.64 (0.03)	0.34 (0.04)

[1]All nutrients expressed as a percent of DM unless otherwise specified.
[2]Amplifier Max (Land O'Lakes Animal Milk Products, Shoreview, MN).
[3]Cow's Match (Land O'Lakes Animal Milk Products).
[4]MomentaCalf (Vita-Plus Corp., Madison, WI).
[5]Long-stem alfalfa/orchardgrass offered free-choice.
[6]Estimated using the equation $ME = 1.01 \times [(0.04409 \times TDN) - 0.45]$.
[7]Estimated using the equation $NE_m = (1.37 \times ME) - (0.138 \times ME^2) + (0.0105 \times ME^3) - 1.12$.
[8]Estimated using the following equation: $NE_g = (1.42 \times ME) - (0.174 \times ME^2) + (0.0122 \times ME^3) - 1.65$.
[9]Non-fiber carbohydrate; calculated as 100 – CP – NDF – Crude Fat – Ash.
[10]Not determined.

Table 2.2. Ingredient and nutrient analysis (± s.d.) of high non-fiber carbohydrate (HNFC) grain mix, low NFC (LNFC) grain mix, and hay fed to weaned heifers and steers from 12 to 28 wk of age[1].

Item	HNFC	LNFC	Hay[2]	Hay[3]
Ingredient, % of DM				
Corn, cracked	--	20.5	--	--
Corn, steam-flaked	51.5	--	--	--
DDGS[4]	10.6	10.6	--	--
Soybean meal	13.6	10.6	--	--
Cottonseed hulls	8.3	22.7	--	--
Soybean hulls	13.6	15.2	--	--
Wheat bran	--	18.2	--	--
Mineral supplement[5]	2.3	2.3	--	--
Nutrient composition[6]				
DM	90.1 (0.5)	91.5 (1.2)	91.8 (0.8)	92.2 (0.8)
ME[7], Mcal/kg	3.11 (0.02)	2.89 (0.09)	2.14 (0.09)	2.11 (0.07)
NE_m[8], Mcal/kg	1.90 (0.03)	1.67 (0.08)	1.08 (0.07)	1.05 (0.05)
NE_g[9], Mcal/kg	1.26 (0.02)	1.06 (0.06)	0.52 (0.07)	0.50 (0.05)
CP	17.3 (1.7)	17.0 (2.0)	17.5 (2.3)	16.6 (1.9)
NFC[10]	51.3 (4.0)	31.3 (4.0)	15.5 (4.5)	12.7 (3.1)
Starch	44.3 (2.5)	19.2 (1.8)	1.2 (0.6)	1.3 (0.7)
NDF	25.6 (2.3)	43.8 (5.8)	55.9 (6.5)	59.9 (4.6)
ADF	16.6 (2.8)	28.3 (4.0)	40.9 (4.4)	39.3 (2.1)
Crude fat	4.1 (0.5)	4.6 (0.5)	1.7 (0.3)	1.8 (0.1)
Ca	0.50 (0.20)	0.60 (0.15)	1.06 (0.24)	0.98 (0.29)
P	0.41 (0.07)	0.60 (0.06)	0.32 (0.03)	0.34 (0.05)

[1]Forage:concentrate ratios during study: 25:75 from 12 to 16 wk; 40:60 from 16 to 24 wk; 55:45 from 24 to 28 wk.
[2]Fed from 12 to 24 wk.
[3]Fed from 24 to 28 wk.
[4]Corn dried distiller's grain with solubles.
[5]Heifer mineral (KNS, Lawrenceburg, KY) containing 1,800 g/ton of monensin (as-fed basis).

[6]All values given as a percent of DM unless otherwise stated.
[7]Estimated using the following equation: $ME = 1.01 \times [(0.04409 \times TDN) - 0.45]$.
[8]Estimated using the following equation: $NE_m = (1.37 \times ME) - (0.138 \times ME^2) + (0.0105 \times ME^3) - 1.12$.
[9]Estimated using the following equation: $NE_g = (1.42 \times ME) - (0.174 \times ME^2) + (0.0122 \times ME^3) - 1.65$.
[10]Calculated using equation NFC = 100 - NDF – CP – Crude fat – Ash.

Table 2.3. Weight and average daily gain (ADG) responses of pre-weaned calves fed conventional milk replacer (CONV) or high nutrition plane milk replacer (HI).

Item	CONV	HI	SEM	*P*-value
Birth weight	43.1	43.9	0.98	--
BW[1,2], kg				
2 wk	47.6	51.5	0.45	< 0.01
4 wk	56.0	62.2	0.60	< 0.01
6 wk	68.4	74.4	0.93	< 0.01
8 wk	81.3	87.4	1.00	< 0.01
11 wk[3]	103.7	108.2	1.21	0.01
Weaning age, d	50	65	1.7	< 0.01
Weaning weight[2,4]	75.5	93.5	1.04	< 0.01
ADG, kg/d				
Birth to 2 wk	0.30	0.56	0.033	< 0.01
Birth to 4 wk	0.41	0.60	0.021	< 0.01
Birth to 6 wk	0.57	0.69	0.017	< 0.01
Birth to weaning	0.66	0.78	0.014	< 0.01
Weaning to 11 wk of age	1.06	1.28	0.066	0.02
Birth to 11 wk of age	0.78	0.84	0.016	0.01

[1]Body weight.
[2]Birth weight included as a covariate in analysis.
[3]Adjusted weight calculated using transport weight from PDREC and ADG from 8 wk to transport.
[4]Calculated from BW and ADG for preceding sample collection period if weaning did not occur on scheduled weigh date.

Table 2.4. Skeletal measurements of pre-weaned calves fed conventional milk replacer (CONV) or high nutrition plane milk replacer (HI) from birth to 12 wk of age.

Item	CONV	HI	SEM	*P*-value
Hip height[1], cm				
Birth[2]	83.7	84.0	0.36	--
4 wk	88.3	89.4	0.36	0.02
8 wk	94.7	97.3	0.36	< 0.01
12 wk	101.5	102.1	0.37	0.24
Total gain, birth to 8 wk	11.0	13.2	0.43	< 0.01
Total gain, 8 to 12 wk	6.3	4.6	0.36	< 0.01
Hip width[1], cm				
Birth[2]	17.7	17.8	0.15	--
4 wk	19.3	20.2	0.15	< 0.01
8 wk	22.4	23.2	0.15	< 0.01
12 wk	24.5	25.9	0.15	0.04
Total gain, birth to 8 wk	4.6	5.4	0.21	< 0.01
Total gain, 8 to 12 wk	2.8	2.6	0.26	0.55
Heart girth[1], cm				
Birth[2]	82.3	82.0	0.49	--
4 wk	89.8	93.7	0.49	< 0.01
8 wk	101.2	104.9	0.49	< 0.01
12 wk	110.9	112.2	0.51	0.08
Total gain, birth to 8 wk	18.9	22.8	0.63	0.02
Total gain, 8 to 12 wk	9.1	7.0	0.64	0.01

[1]Treatment×time interaction significant at $P < 0.01$ level.
[2]Starting measurement included as a covariate.

Table 2.5. Intake responses of pre-weaned calves fed conventional milk replacer (CONV) or high nutrition plane milk replacer (HI).

Item[1]	CONV	HI	SEM	*P*-value
Weaning age, d	50	65	1.7	< 0.01
Feed intake to weaning, kg				
Total MR[2]	32.7	65.7	1.58	< 0.01
Total starter	25.6	27.3	1.01	0.18
Total DM	58.4	92.8	2.11	< 0.01
ME intake to weaning, Mcal				
Total MR	143.2	298.7	7.64	< 0.01
Total starter	86.8	92.4	3.43	0.20
Total DM	229.9	390.9	9.34	< 0.01
CP intake to weaning, kg				
Total MR	7.4	19.0	0.44	< 0.01
Total starter	6.2	6.6	0.25	0.22
Total DM	13.6	25.6	0.58	< 0.01
Total DM intake, kg				
Birth to 11 wk of age[3]	165.2	161.6	10.63	0.60
Weaning to 11 wk of age[3]	106.5	68.3	10.72	< 0.01
Feed efficiency[4]				
Birth to weaning[5]	0.533	0.557	0.011	0.16
Weaning to 11 wk of age[3]	0.314	0.309	0.021	0.80
Birth to 11 wk of age[3,5]	0.407	0.427	0.018	0.23

[1]Values given in kg unless otherwise specified.
[2]Milk replacer.
[3]Calf sex included as covariate in model.
[4]Total BW gain/total DM intake.
[5]Weaning age included as covariate in model.

Table 2.6. Rumen fermentation parameters at 11 wk of age of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning.

Item	CONV	HI	SEM	*P*-value
Rumen pH	5.57	5.53	0.071	0.58
Rumen NH3, mg/dL	11.3	9.3	1.34	0.24
Total VFA[1], mM	116.3	101.7	5.52	0.06
Molar proportion of VFA[2]				
Acetate	48.3	47.5	1.17	0.52
Propionate	33.0	32.8	1.28	0.88
Butyrate	12.2	12.4	0.83	0.87
Valerate	4.8	5.7	0.55	0.25
Isobutyrate	0.6	0.5	0.05	0.26
Isovalerate	1.0	1.1	0.14	0.87
Isoacids[3]	1.6	1.6	0.17	0.86
A:P[4]	1.50	1.53	0.100	0.73

[1]Volatile fatty acids.
[2]Molar proportion expressed as mol individual VFA/100 mol total VFA.
[3]Sum of isovalerate and isobutyrate molar proportions.
[4]Acetate:propionate ratio.

Table 2.7. Health measurements of pre-weaned calves fed conventional milk replacer (CONV) or high nutrition plane milk replacer (HI).

Item	CONV	HI	SEM	*P*-value
Total protein, mg/dL[1]	7.01	7.03	0.15	0.91
Health scores[2]				
Fecal	1.4	1.5	0.03	0.03
0 to 2 wk	1.9	2.1	0.07	< 0.01
2 to 4 wk	1.4	1.7	0.08	< 0.01
4 to 6 wk	1.1	1.2	0.03	< 0.01
6 to 8 wk	1.2	1.2	0.06	0.62
Respiratory	1.0	1.0	0.01	0.78
General appearance	1.0	1.0	0.01	0.19
Scour days[3]	4.4	7.4	0.82	< 0.01

[1]Measured within 3 d of age.
[2]1 to 5 system for each parameter as described by Heinrichs et al. (2003).
[3]Scour day defined as fecal score ≥ 3.

Table 2.8. Skeletal growth of Holstein heifers and steers fed high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning diets from 12 to 28 wk of age.

Item	HNFC	LNFC	SEM	*P*-value
BW[1,2], kg				
12 wk	110.0	111.0	1.65	--
16 wk	139.2	137.1	2.15	0.50
20 wk	170.5	166.2	2.15	0.15
24 wk	202.5	194.9	2.15	0.01
28 wk	235.6	225.9	2.15	< 0.01
ADG[3], kg/d	1.12	1.03	0.030	0.04
Hip height, cm				
12 wk[2]	102.2	102.2	0.41	--
28 wk	121.3	120.3	0.41	0.06
Total gain[4]	20.1	19.0	0.59	0.07
Withers height, cm				
12 wk[2]	98.3	98.3	0.40	--
28 wk	117.1	116.3	0.40	0.13
Total gain[4]	19.5	18.7	0.59	0.13
Hip width, cm				
12 wk[2]	25.5	25.6	0.23	--
28 wk	35.4	34.9	0.23	0.08
Total gain	10.0	9.3	0.27	0.01
Heart girth, cm				
12 wk[2]	112.0	112.0	0.60	--
28 wk	143.9	143.6	0.60	0.70
Total gain	31.9	31.6	0.74	0.70
BCS[5], 1 to 5 scale				
12 wk[2]	2.74	2.72	0.040	--
28 wk	3.12	3.03	0.040	0.13
Total gain	0.38	0.31	0.048	0.29

[1]Body weight.
[2]Starting measurement at 12 wk included as covariate in analysis.
[3]Average daily gain.
[4]Sex effect significant at $P \leq 0.05$ level.
[5]Body condition score.

Table 2.9. Growth, intake, and efficiency responses of Holstein heifers and steers fed high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning diets from 12 to 28 wk of age.

				P-value[1]	
Item	HNFC	LNFC	SEM	T	T×S
Total DMI[2], kg/d	5.97	6.24	0.250	0.31	0.14
Total DMI, % of BW	3.32	3.55	0.084	0.04	0.17
ME intake, Mcal/d	16.1	16.0	0.58	0.93	< 0.01
ME intake, Mcal/100 kg BW	8.89	9.08	0.202	0.50	0.03
CP intake, kg/d	1.0	1.1	0.04	0.28	< 0.01
CP intake, % of BW	0.56	0.60	0.013	< 0.01	0.02
NDFI[3], kg/d	2.4	3.1	0.10	< 0.01	< 0.01
NDFI, % of BW	1.28	1.73	0.038	< 0.01	0.06
fNDFI[4] intake, kg/d	1.5	1.5	0.05	0.85	< 0.01
fNDFI, % of BW	0.76	0.79	0.019	0.18	0.58
Feed efficiency[5]					
Overall average	0.205	0.179	0.006	< 0.01	0.06
12 wk to 16 wk	0.272	0.230	0.008	< 0.01	--
16 wk to 24 wk	0.195	0.167	0.008	0.01	--
24 wk to 28 wk	0.148	0.141	0.008	0.48	--
ME efficiency[6]					
Overall average	0.074	0.068	0.002	0.02	0.21
CP efficiency[7]					
Overall average	1.20	1.03	0.032	< 0.01	0.03
12 wk to 16 wk	1.58	1.29	0.050	< 0.01	--
16 wk to 24 wk	1.13	0.96	0.050	0.02	--
24 wk to 28 wk	0.89	0.85	0.050	0.54	--

[1]T = treatment effect; T×S = treatment by time interaction.
[2]Dry matter intake.
[3]NDF intake.
[4]Forage NDF intake.
[5]Expressed as kg of ADG/kg daily DMI.
[6]Expressed as kg of ADG/Mcal of daily ME intake.
[7]Expressed as kg of ADG/kg daily CP intake.

Table 2.10. Blood metabolites and rumen fermentation parameters of dairy calves fed high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning diets from 12 to 28 wk of age.

				P-value[1]	
Item	HNFC	LNFC	SEM	T	T×S
Glucose, mg/dL	87.7	83.3	1.40	0.02	0.86
PUN[2], mg/dL	7.6	8.9	0.20	< 0.01	0.23
Rumen pH	6.23	6.24	0.04	0.91	0.10
Rumen NH3, mg/dL	4.9	10.3	0.40	< 0.01	0.02
Total VFA[3], mM	106.9	104.2	3.19	0.52	0.12
Molar proportion of VFA[4]					
Acetate	63.1	66.5	0.56	< 0.01	0.04
Propionate	25.2	22.7	0.50	< 0.01	< 0.01
Butyrate	8.4	7.9	0.22	0.08	0.37
Valerate	1.5	1.4	0.05	0.25	0.22
Isobutyrate	0.5	0.4	0.03	0.08	0.15
Isovalerate	1.3	1.1	0.08	0.03	0.32
Isoacids[5]	1.9	1.5	0.10	0.02	0.28
A:P[6]	2.76	3.03	0.07	< 0.01	0.05

[1]T = treatment effect; T×S = treatment by time interaction.
[2]Plasma urea N.
[3]Volatile fatty acids.
[4]Molar proportion expressed as mol individual VFA/100 mol total VFA.
[5]Sum of isovalerate and isobutyrate molar proportions.
[6]Acetate:propionate ratio.

Table 2.11. Weight, skeletal growth, and feed efficiency responses of dairy calves previously fed conventional milk replacer (CONV) or high nutrition plane milk replacer (HI) and fed high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning grower diets from 12 to 28 wk of age.

	CONV		HI			*P*-value[1]		
Item	HNFC	LNFC	HNFC	LNFC	SEM	Pre	Post	Pre×Post
BW[2], kg								
12 wk	108.4	110.1	112.2	111.4	3.62	0.43	--	--
28 wk	234.2^{a}	228.1^{ab}	236.8^{a}	224.4^{b}	3.62	0.88	< 0.01	0.04
ADG, kg/d								
Birth to 12 wk	0.75	0.75	0.78	0.77	0.035	0.35	--	--
12 to 28 wk	1.12^{a}	1.05^{ab}	1.11^{a}	1.01^{b}	0.035	0.40	< 0.01	0.05
Birth to 28 wk	0.96	0.92	0.97	0.90	0.024	0.95	0.01	0.49
Feed efficiency								
12 to 16 wk	0.257^{b}	0.237^{bc}	0.287^{a}	0.222^{c}	0.011	0.48	< 0.01	< 0.01
16 to 24 wk	0.200^{a,x}	0.171^{ab,y}	0.191^{a}	0.162^{b}	0.011	0.38	0.01	0.06
24 to 28 wk	0.150	0.141	0.147	0.140	0.011	0.89	0.48	0.91
Overall	0.202	0.183	0.208	0.175	0.007	0.87	< 0.01	0.31
ME efficiency								
12 to 16 wk	0.089^{ab,y}	0.086^{b}	0.099^{a,x}	0.081^{b}	0.004	0.51	0.01	0.02
16 to 24 wk	0.073	0.066	0.070	0.062	0.004	0.38	0.07	0.25
24 to 28 wk	0.058	0.057	0.057	0.057	0.004	0.87	0.83	0.99
Overall	0.073	0.070	0.075	0.067	0.003	0.84	0.02	0.32
Hip height at 28 wk[2], cm	120.9	119.8	120.6	120.3	0.67	0.88	0.25	0.54
Hip width at 28 wk[2], cm	35.2^{ab}	35.4^{ab,x}	35.7^{a}	34.9^{b,y}	0.32	0.99	0.26	0.08
Heart girth at 28 wk[2], cm	142.7	143.4	143.9	143.4	1.31	0.55	0.93	0.56
BCS at 28 wk, 1 to 5 scale	3.12	2.99	3.15	3.04	0.07	0.45	0.05	0.91

[1]Pre = effect of pre-weaning diet; Post = effect of post-weaning diet; Pre×Post = interaction of pre- and post-weaning diet effects.
[2]Measurements at birth included in model as a covariate.
[abc]Means with differing superscripts significantly differ at $P \leq 0.05$ level.
[xy]Means with differing superscripts tend to differ at $0.10 \geq P > 0.05$ level.

Table 2.12. Rumen fermentation parameters at harvest of 12 wk-old bulls fed a conventional (CONV) or high (HI) plane of nutrition pre-weaning and 28 wk-old steers fed high non-fiber carbohydrate (HNFC) or low NFC (LNFC) grower diet post-weaning.

Item	CONV	HI	SEM	*P*-value	HNFC	LNFC	SEM	*P*-value
n	3	3	--	--	6	6	--	--
Rumen pH	5.06	5.39	0.13	0.22	6.15	6.51	0.17	0.08
Rumen NH3, mg/dL	21.20	14.62	4.89	0.44	8.98	6.05	2.85	0.27
Total VFA[1], mM	196.9	143.8	15.52	0.14	109.4	96.4	6.71	0.20
Molar proportion of VFA[2]								
Acetate	49.34	49.31	0.74	0.95	68.44	75.12	1.48	0.01
Propionate	36.94	41.70	1.33	0.07	20.53	15.78	0.99	< 0.01
Butyrate	8.53	5.78	0.36	< 0.01	6.48	4.80	0.40	< 0.01
Valerate	4.32	2.40	0.70	0.19	1.00	1.04	0.06	0.67
Isobutyrate	0.41	0.37	0.04	0.55	1.17	1.19	0.05	0.82
Isovalerate	0.46	0.45	0.07	0.91	2.38	2.07	0.28	0.10
Isoacids[3]	0.86	0.82	0.09	0.63	3.55	3.26	0.26	0.20
A:P[4]	1.34	1.19	0.06	0.08	3.41	4.83	0.27	< 0.01

[1]Volatile fatty acids.
[2]Expressed as mol/100 mol of total VFA.
[3]Sum of isobutyrate and isovalerate molar proportions.
[4]Acetate:propionate ratio.

Table 2.13. Harvest weights and histological papillae morphology of 12 wk-old bulls fed a conventional (CONV) or high (HI) plane of nutrition pre-weaning and 28 wk-old steers fed high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning diets.

Item[1]	CONV	HI	SEM	*P*-value	HNFC	LNFC	SEM	*P*-value
n	3	3	--	--	6	6	--	--
Live weight	105.2	117.8	5.84	0.17	244.2	228.7	9.24	0.10
Hot carcass weight	55.1	61.5	3.04	0.21	129.0	118.8	5.46	0.06
Dressing %	52.4	52.2	0.46	0.79	52.8	52.0	0.53	0.30
Full foregut weight	13.8	14.4	0.76	0.62	37.9	35.1	2.88	0.39
Full reticulorumen weight	11.8	12.3	0.53	0.56	31.9	28.5	2.46	0.27
Empty reticulorumen weight	2.4	2.3	0.23	0.73	6.3	6.4	0.44	0.92
Full rumen:total foregut[2]	85.6	85.6	1.03	> 0.99	84.3	80.7	1.10	0.05
Full rumen:LW[2]	11.2	10.4	0.28	0.20	13.3	12.3	0.98	0.42
Empty rumen:LW[2]	2.3	2.0	0.11	0.13	2.6	2.8	0.26	0.29
Empty rumen:HCW[2]	4.4	3.7	0.20	0.13	5.0	5.4	0.53	0.25
Mucosa:ERW[3]	50.1	47.1	3.28	0.50	50.2	49.9	2.45	0.88
Mucosa:LW[3]	1.15	0.92	0.072	0.03	1.31	1.37	0.79	0.30
Mucosa:HCW[3]	2.21	1.75	0.139	0.03	2.49	2.64	0.17	0.18
Muscle:ERW[3]	46.3	47.5	2.95	0.77	48.1	49.0	2.09	0.53
Muscle:LW[3]	1.07	0.92	0.074	0.18	1.28	1.37	0.18	0.13
Muscle:HCW[3]	2.05	1.77	0.144	0.18	2.43	2.64	0.36	0.09
Papillae length[a], mm	1.92	2.00	0.210	0.66	2.87	3.42	0.282	0.05
Papillae width[b], mm	0.50	0.47	0.032	0.33	0.46	0.50	0.049	0.42
Surface area[b], mm^2	3.56	3.22	0.497	0.40	5.09	6.44	1.300	0.27

[1]Values given in kg unless otherwise stated.
[2]Ratio of full or empty reticulorumen weight (ERW) to live weight (LW) or hot carcass weight (HCW) expressed as a percent.
[3]Ratio of mucosal or muscle tissue to ERW, LW, or HCW expressed as a percent on a wet tissue basis.
[a]Post-weaning treatment×sample location interaction; $P \leq 0.05$ for cranial ventral samples only.
[b]Pre-weaning treatment×sample location interaction; $P \leq 0.10$ for caudal ventral samples only.

Table 2.14. Harvest weights and histological papillae morphology of 28 wk-old Holstein steers previously fed conventional milk replacer (CONV) or high nutrition plane milk replacer (HI) and fed high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning grower diets from 12 to 28 wk of age.

	CONV		HI			*P*-value[1]		
Item[2]	HNFC	LNFC	HNFC	LNFC	SEM	Pre	Post	Pre×Post
n	3	3	3	3	--	--	--	--
Live weight	235.5	231.0	253.5	225.8	10.81	0.44	0.09	0.21
Hot carcass weight	123.5	117.4	134.7	120.0	6.02	0.12	0.04	0.32
Dressing %	52.3	50.8	53.3	53.1	0.56	0.02	0.14	0.24
Full foregut weight	37.4	39.1	38.4	31.2	3.35	0.24	0.34	0.14
Full reticulorumen weight	31.4	31.9	32.4	25.2	3.02	0.31	0.24	0.19
Empty reticulorumen weight	6.2	6.6	6.5	6.2	0.54	0.96	0.93	0.40
Full rumen:total foregut[3]	84.0	81.7	84.5	79.7	1.66	0.67	0.07	0.48
Full rumen:LW[3]	13.4	13.9	13.2	10.7	1.12	0.10	0.32	0.14
ERW:LW[3]	2.6	2.9	2.6	2.7	0.27	0.41	0.30	0.36
ERW:HCW[3]	5.1	5.7	5.0	5.0	0.56	0.20	0.23	0.30
Mucosa:ERW[4]	52.1	48.0	48.3	51.9	2.77	> 0.99	0.88	0.05
Mucosa:LW[4]	1.4	1.4	1.3	1.4	0.09	0.39	0.32	0.47
Mucosa:HCW[4]	2.6	2.7	2.4	2.6	0.18	0.12	0.19	0.67
Muscle:ERW[4]	46.3	49.4	49.9	48.7	2.35	0.32	0.53	0.17
Muscle:LW[4]	1.2[b]	1.4[a]	1.3[ab]	1.3[ab]	0.19	0.72	0.13	0.05
Muscle:HCW[4]	2.4[b]	2.8[a]	2.5[b]	2.5[b]	0.37	0.30	0.08	0.03
Papillae length, mm	3.09	3.56	2.64	3.26	0.362	0.20	0.07	0.79
Papillae width, mm	0.47	0.52	0.45	0.48	0.067	0.57	0.45	0.83
Surface area, mm^2	5.45	7.43	4.73	5.53	1.680	0.33	0.30	0.65

[1]Pre = effect of pre-weaning diet; Post = effect of post-weaning diet; Pre×Post = interaction of main effects.
[2]Values given in kg unless otherwise stated.
[3]Ratio of full or empty reticulorumen weight (ERW) to live weight (LW) or hot carcass weight (HCW) expressed as a percent.
[4]Ratio of mucosal or muscle tissue to empty ERW, LW, or HCW expressed as a percent on a wet tissue basis.
[ab]$P \leq 0.05$.

Table 2.15. Hand measurements of rumen papillae in cranial and caudal ventral regions of the reticulorumen from 12- and 28-wk old male dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning.

	12 wk		28 wk			*P*-value[1]		
Item	CONV	HI	CONV	HI	SEM	Trt	Age	Pre×Age
n	3	3	6	6	--	--	--	--
Papillae length, mm								
Cranial	4.3^{cd}	4.5^{c}	9.8^{a}	9.3^{a}	0.49	0.68	< 0.01	< 0.01
Caudal	4.1^{cd}	3.5^{d}	7.2^{b}	6.8^{b}	0.49	0.22	< 0.01	< 0.01
Papillae width, mm								
Cranial	1.9	1.7	2.2	2.0	0.20	0.15	0.14	0.18
Caudal	1.7^{b}	1.6^{b}	2.3^{a}	2.3^{a}	0.20	0.71	< 0.01	0.01
Surface area[2], mm^2								
Cranial	12.9^{c}	12.3^{c,x}	34.8^{a}	28.9^{b}	2.89	0.09	< 0.01	< 0.01
Caudal	10.9^{c}	9.1^{c,y}	27.1^{b}	25.1^{b}	2.94	0.33	< 0.01	< 0.01
Papillae density[3]								
Cranial	81.0^{a}	69.7^{a}	46.9^{c}	50.5^{bc}	7.8	0.55	< 0.01	0.02
Caudal	79.7^{a}	84.5^{a}	50.3^{bc}	48.3^{bc}	7.8	0.82	< 0.01	< 0.01
Surface area ratio[4]								
Cranial	1.61^{c}	1.81^{c}	7.81^{a}	6.01^{b}	1.09	0.27	< 0.01	< 0.01
Caudal	1.44^{c}	1.14^{c}	5.53^{b}	5.62^{b}	1.09	0.88	< 0.01	0.03

[1]Trt = pre-weaning treatment effect; Age = age at harvest; Trt×Age = interaction of main effects.
[2]Calculated using the following equation: Surface area (mm^2) = 2 × [(length / 2) × (width / 2) × π].
[3]Number of papillae per cm^2.
[4]Calculated using the following equation: Surface area ratio (cm^2 / cm^2) = Surface area / papillae density.
[abcd]Means with differing superscripts in rows and/or columns differ at $P \leq 0.05$ level; Pre × age × region interaction ($P \leq 0.10$).
[xy]Means with differing superscripts within a column tend to differ at $P \leq 0.10$ level; Pre × age × region interaction ($P \leq 0.10$).

Table 2.16. Hand measurements of rumen papillae in cranial and caudal ventral regions of the reticulorumen from 28-wk old male dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning and low NFC (LNFC) or high NFC (HNFC) diets post-weaning.

	CONV		HI			*P*-value[1]		
Item	LNFC	HNFC	LNFC	HNFC	SEM	Trt	Post	Pre×Post
n	3	3	3	3	--	--	--	--
Papillae length, mm								
Cranial	9.7	9.9	9.3	9.9	0.53	0.27	0.82	0.72
Caudal	6.7	7.7	6.6	7.0	0.53	0.40	0.14	0.36
Papillae width, mm								
Cranial	2.3	2.2	2.1	1.9	0.22	0.13	0.36	0.35
Caudal	$2.4^{ab,x}$	2.3^{ab}	2.6^{a}	$2.0^{b,y}$	0.22	0.70	0.04	0.10
Surface area[2], mm^2								
Cranial	34.7	34.8	29.8	28.0	3.64	0.02	0.75	0.15
Caudal	26.0	28.2	27.0	23.3	3.61	0.46	0.77	0.59
Papillae density[3]								
Cranial	45.5	48.3	50.3	50.7	6.7	0.54	0.79	0.91
Caudal	40.7	59.8	44.7	52.0	6.7	0.74	0.05	0.16
Surface area ratio[4]								
Cranial	8.40	7.22	6.26	5.76	1.39	0.09	0.40	0.28
Caudal	6.30	4.77	6.18	5.07	1.39	0.92	0.20	0.59

[1]Trt = pre-weaning treatment effect; Post = post-weaning treatment effect; Trt×Post = interaction of main effects.
[2]Calculated using the following equation: Surface area (mm^2) = 2 × [(length / 2) × (width / 2) × π].
[3]Number of papillae per cm^2.
[4]Calculated using the following equation: Surface area ratio (cm^2 / cm^2) = Surface area / papillae density.
[ab]Means with differing superscripts in a row differ at $P \leq 0.05$ level.
[xy]Means with differing superscripts within a row tend to differ at $P \leq 0.10$ level.

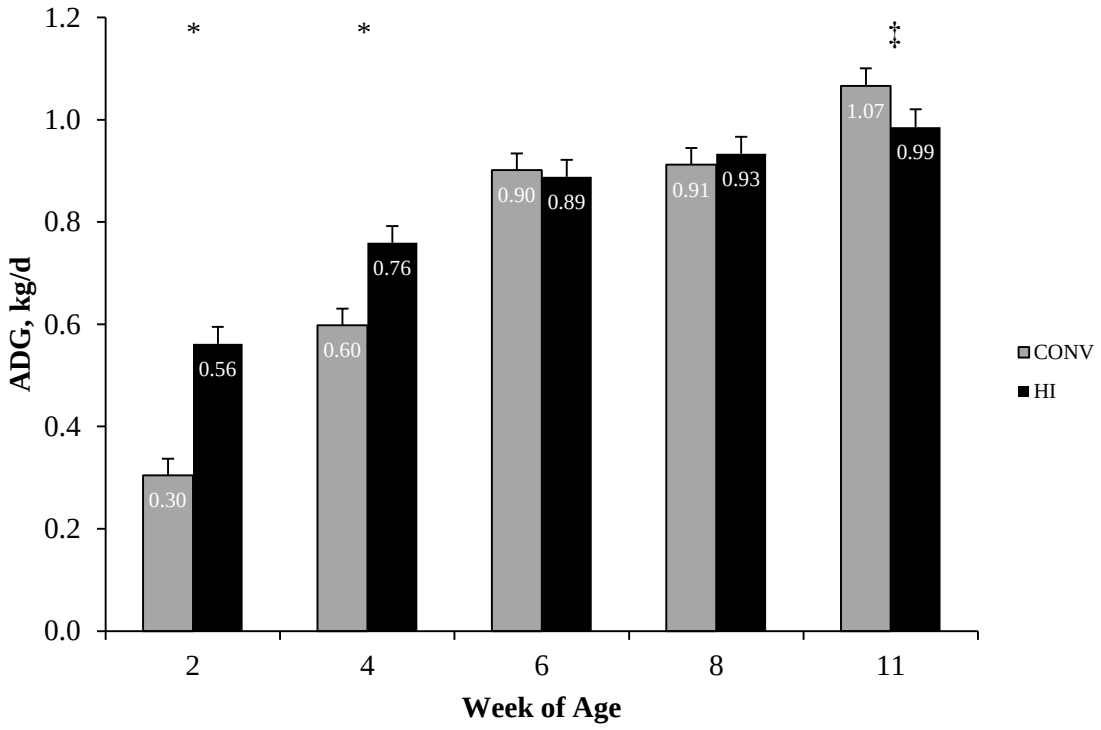

Figure 2.1. Average daily gain (ADG) of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. A treatment×time interaction was observed as calves fed HI had greater ADG from birth to 4 wk of age ($P < 0.01$); however, ADG were similar between treatments thereafter and tended to be greater for calves fed CONV from 8 to 11 wk of age ($P = 0.10$). ‡$0.10 \leq P < 0.05$; *$P \leq 0.01$.

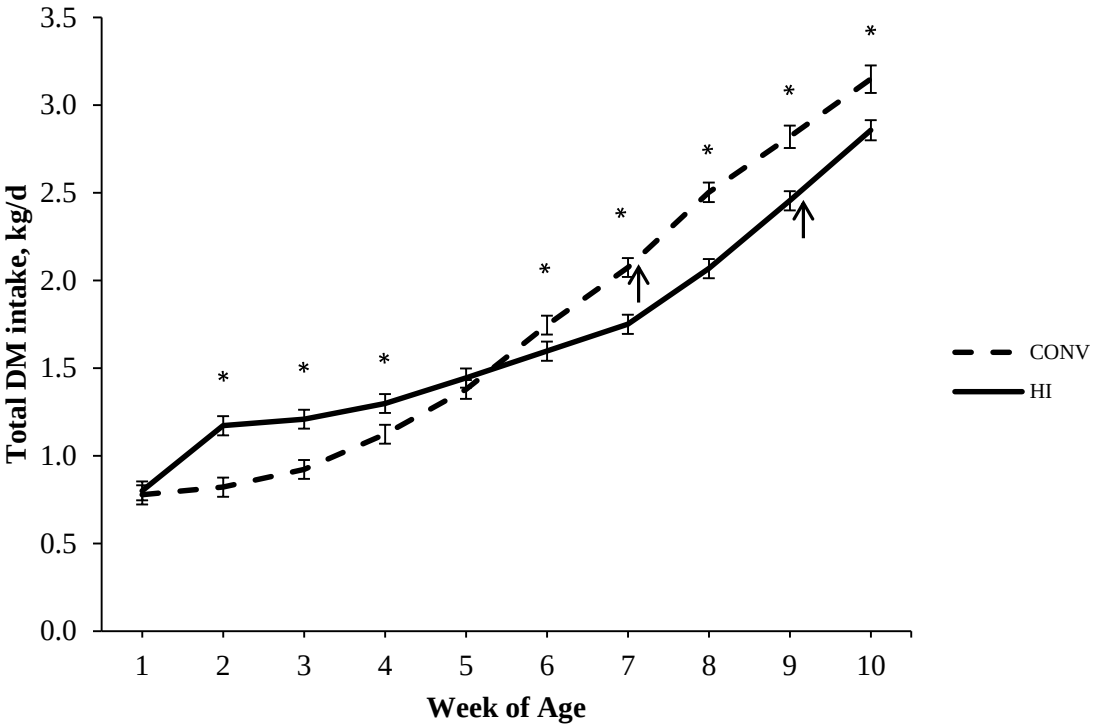

Figure 2.2. Total DM intake of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. Vertical arrows indicate average d of weaning for each treatment (50 d for CONV and 65 d for HI). A treatment×time interaction was observed ($P < 0.01$) as calves fed CONV consumed less total DM at 2, 3, and 4 wk of age compared to calves fed HI, but more total DM after 6 wk of age ($P \leq 0.01$). Overall effect of treatment was not significant ($P = 0.21$). *$P \leq 0.01$.

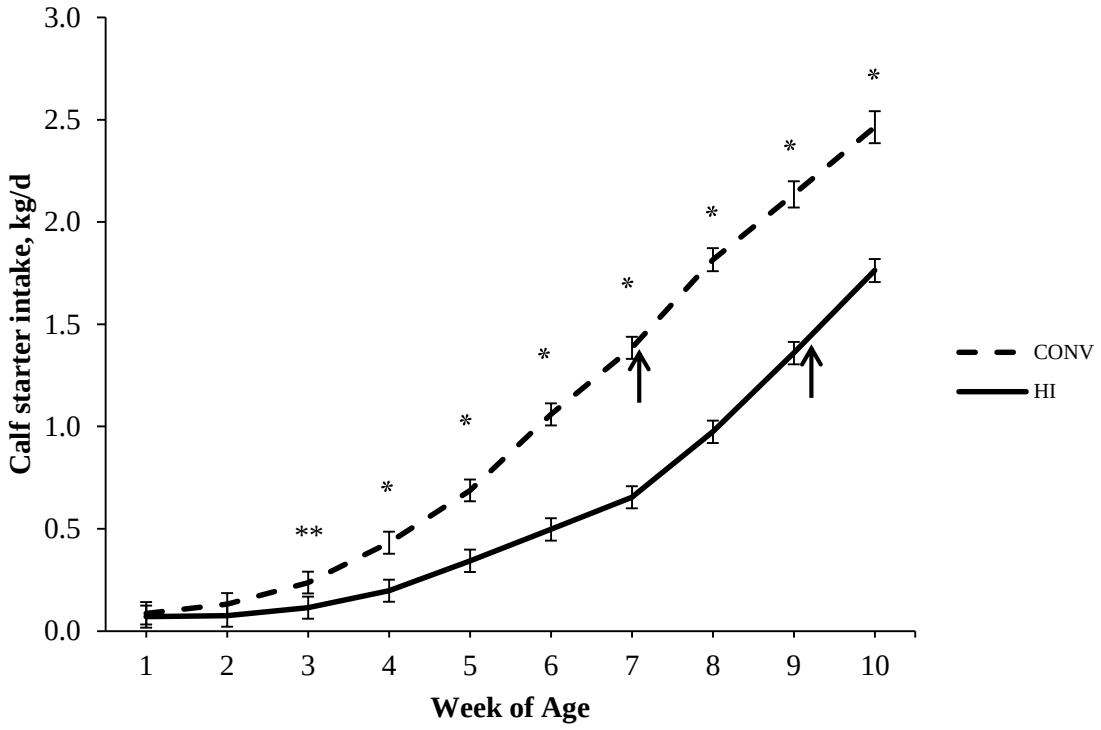

Figure 2.3. Starter intake (DM basis) of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. Vertical arrows indicate average d of weaning for each treatment (50 d for CONV and 65 d for HI). A treatment×time interaction was observed ($P < 0.01$) as calves fed CONV consumed starter more rapidly than calves fed HI, with differences in intake observed beginning at 3 wk of age ($P = 0.03$). **$0.05 \leq P < 0.01$; *$P \leq 0.01$.

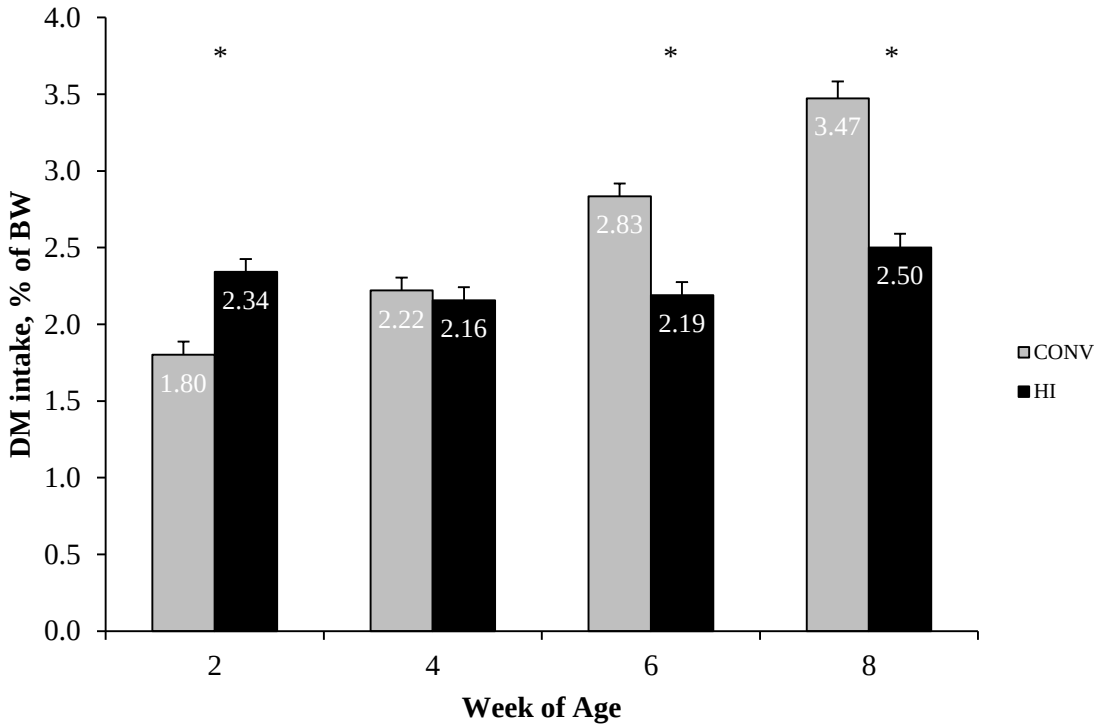

Figure 2.4. Effects of feeding conventional (CONV) or high (HI) planes of nutrition pre-weaning to dairy calves on total DM intake as a percent of body weight (% of BW). A treatment×time interaction was observed ($P < 0.01$) as calves fed HI consumed significantly more DM at 2 wk of age ($P < 0.01$), but intake was similar between treatments at 4 wk of ($P = 0.59$) and steadily increased for calves fed CONV from 2 to 8 wk of age. *$P \leq 0.01$.

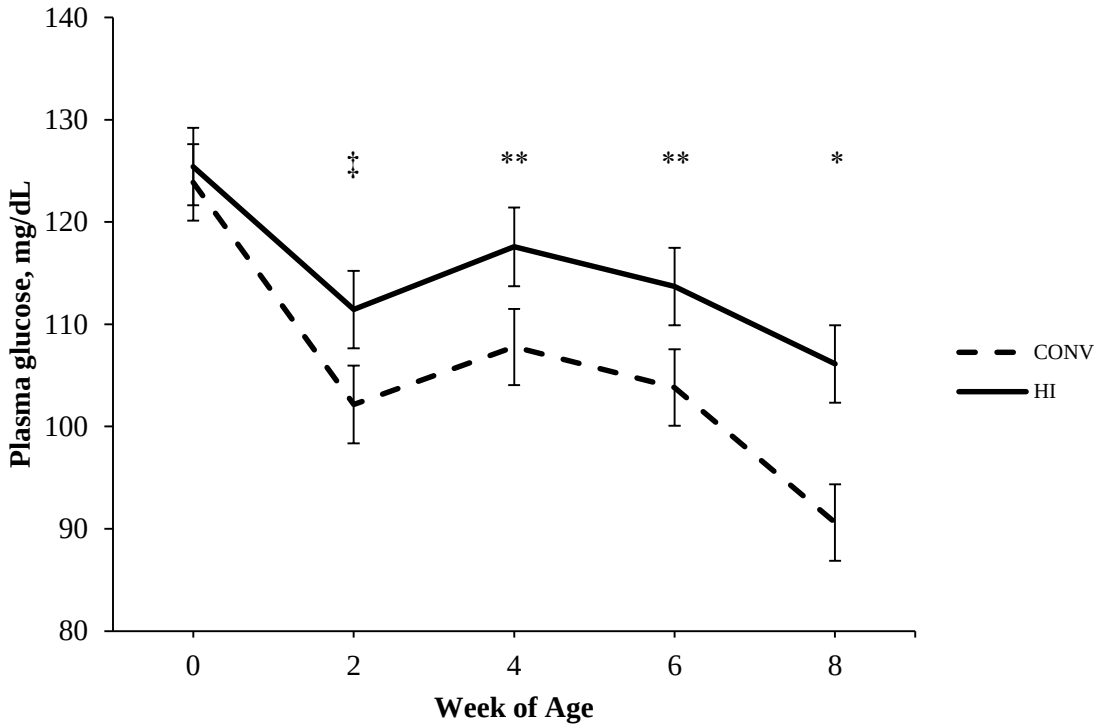

Figure 2.5. Plasma glucose concentrations of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. A treatment effect was observed ($P < 0.01$) as calves fed HI exhibited greater plasma glucose concentrations from birth to 8 wk of age compared to calves fed CONV. ‡$0.10 \leq P < 0.05$; **$0.05 \leq P < 0.01$; *$P \leq 0.01$.

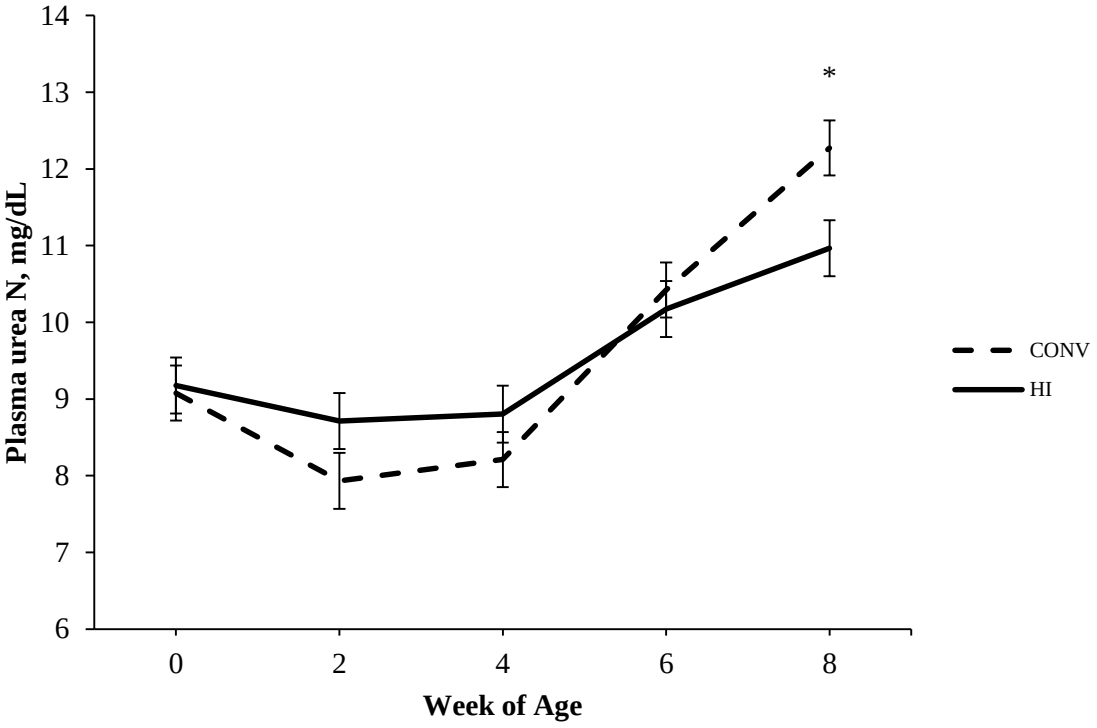

Figure 2.6. Plasma urea N (PUN) concentrations of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. No overall effect of treatment was observed ($P = 0.95$). However, a treatment×time interaction was observed ($P = 0.02$) as PUN were similar between treatments from birth to 6 wk of age, but were elevated for calves fed CONV compared to HI at 8 wk of age ($P = 0.01$). *$P \leq 0.01$.

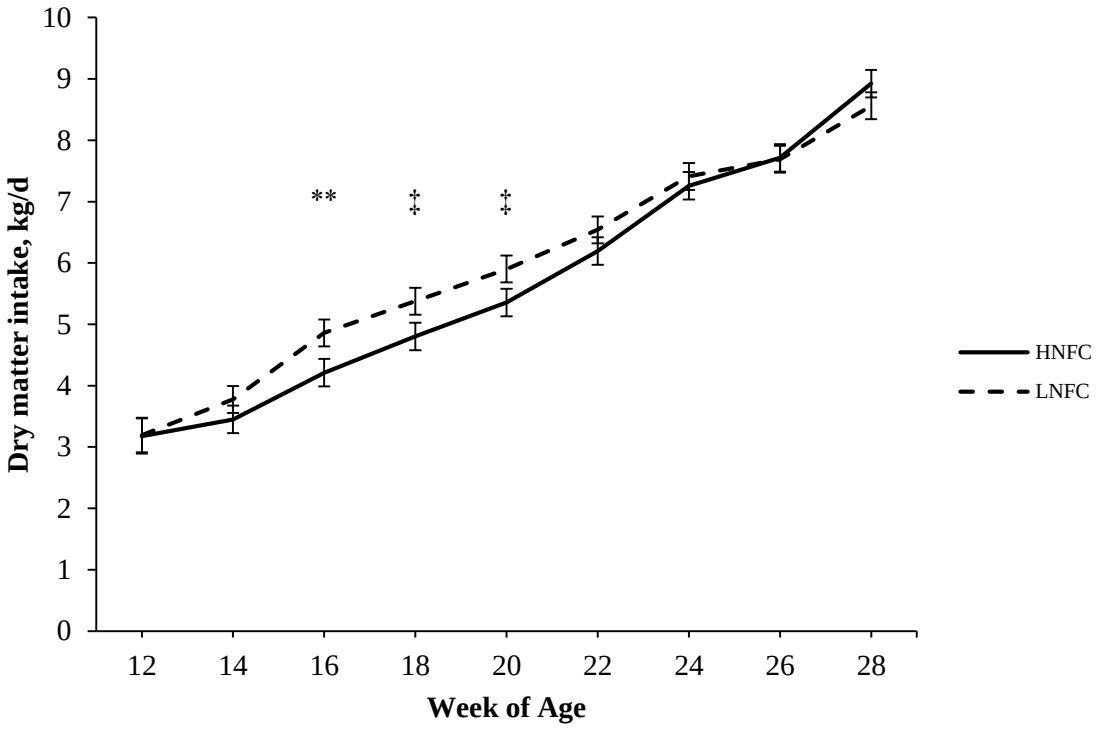

Figure 2.7. Dry matter intake of calves fed a low NFC (LNFC) or high NFC (HNFC) diet from 12 to 28 wk of age. A treatment×time interaction ($P < 0.01$) was observed. Intake diverges from 12 to 20 wk of age but converges when hay inclusion increased from 25 to 40% of the diet after 20 wk of age. No overall effect of NFC was observed ($P = 0.42$). ‡$0.10 \leq P < 0.05$; **$P \leq 0.05$.

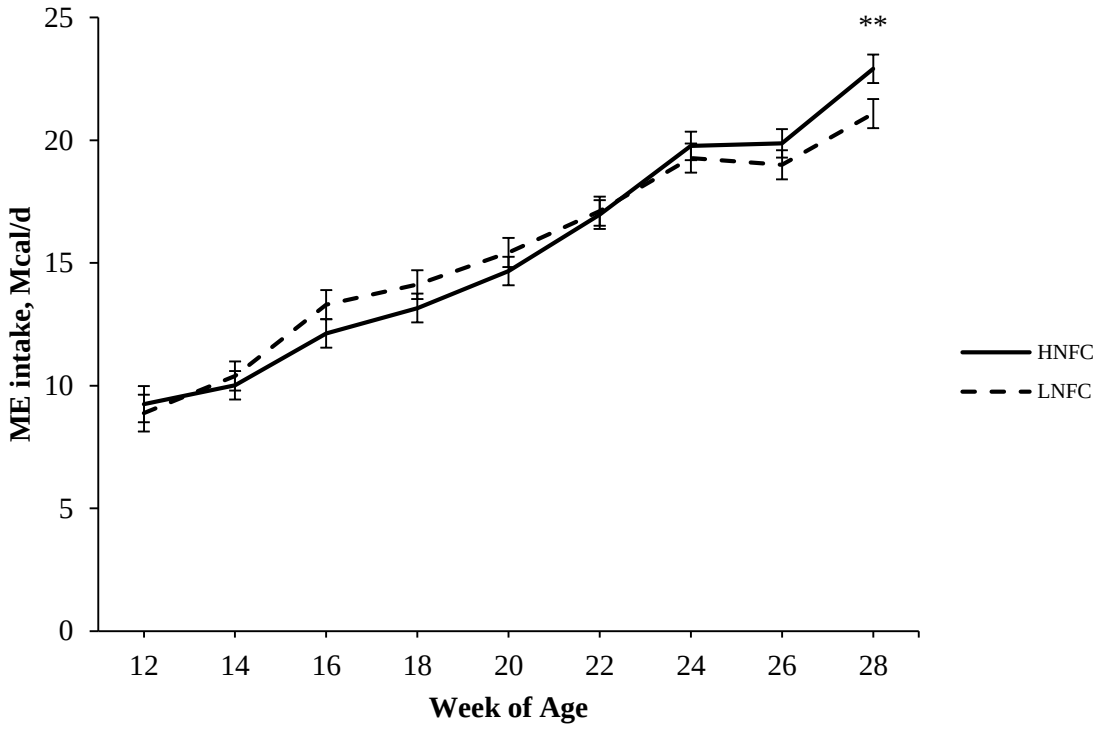

Figure 2.8. Daily intake of ME for weaned calves fed low NFC (LNFC) or high NFC (HNFC) diets from 12 to 28 wk of age. A treatment×time interaction was observed ($P < 0.01$) as intakes were similar throughout the trial but differed at 28 wk of age ($P = 0.03$) due to similar DM intake. $^{**}P \leq 0.05$.

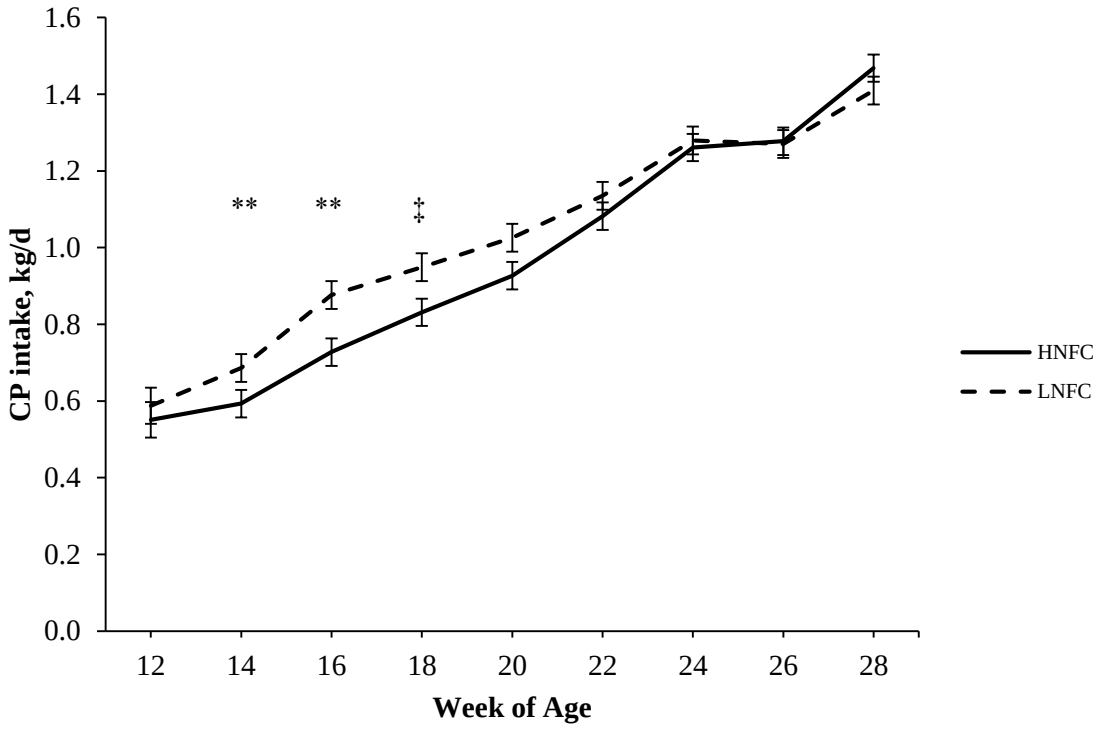

Figure 2.9. Daily intake of CP for weaned calves fed low NFC (LNFC) or high NFC (HNFC) diets from 12 to 28 wk of age. A tendency for a treatment×time interaction was observed ($P = 0.06$), as intakes were greatest for LNFC from 14 to 18 wk of age, but converged thereafter. No overall effect of NFC was observed ($P = 0.28$). ‡$0.10 \leq P < 0.05$. **$P \leq 0.05$.

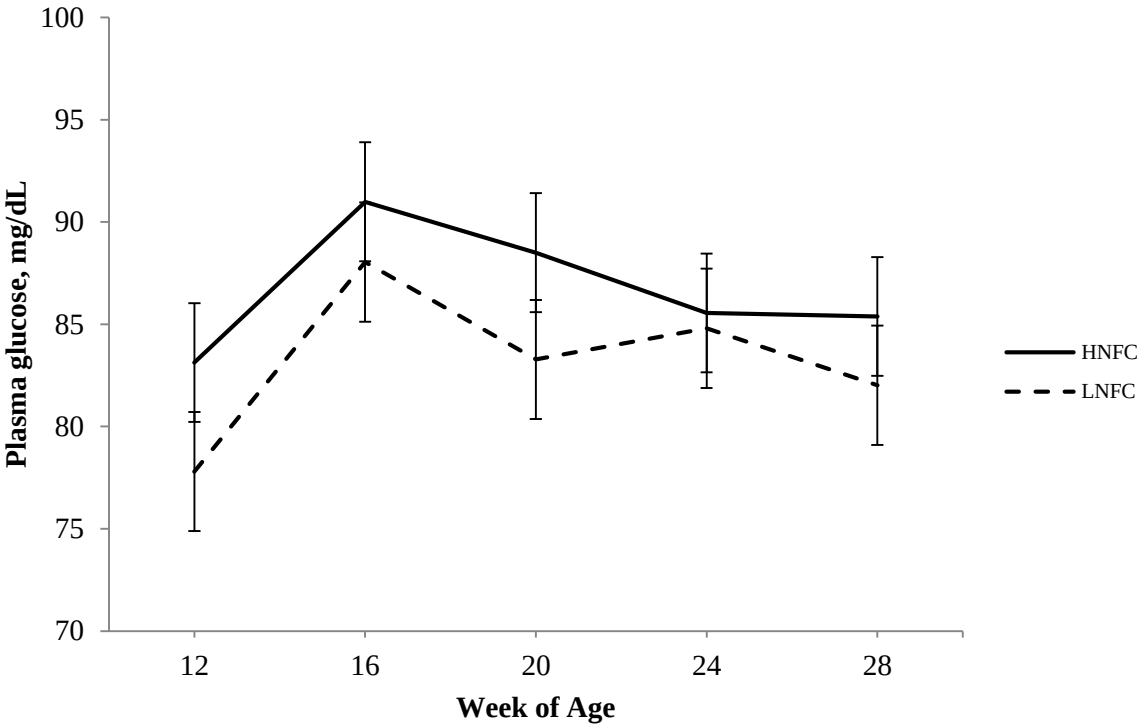

Figure 2.10. Plasma glucose concentrations of calves fed diets containing low non-fiber carbohydrate (LNFC) or high NFC (HNFC) from 12 to 28 wk of age. Concentrations at 12 wk of age were included in the model as a covariate. An overall treatment effect was observed ($P = 0.02$) as calves receiving HNFC diets exhibited greater glucose concentrations from 12 to 28 wk of age. A sex×time effect was also observed as glucose was elevated at wk 24 and wk 28 for steers compared to heifers ($P \leq 0.01$).

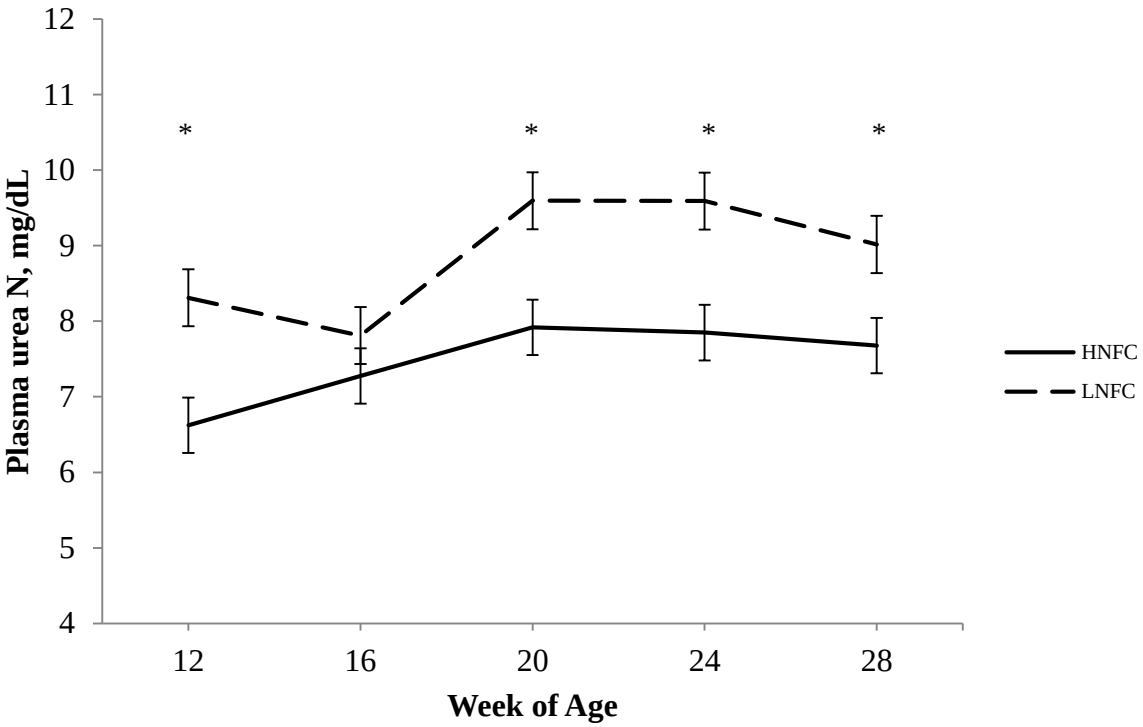

Figure 2.11. Plasma urea N (PUN) of calves fed diets containing low non-fiber carbohydrate (LNFC) or high NFC (HNFC) from 12 to 28 wk of age. Concentrations at 12 wk of age were included in the model as a covariate. An overall treatment effect was observed ($P < 0.01$) as calves receiving LNFC diets exhibited greater PUN concentrations from 12 to 28 wk of age. *$P \leq 0.01$.

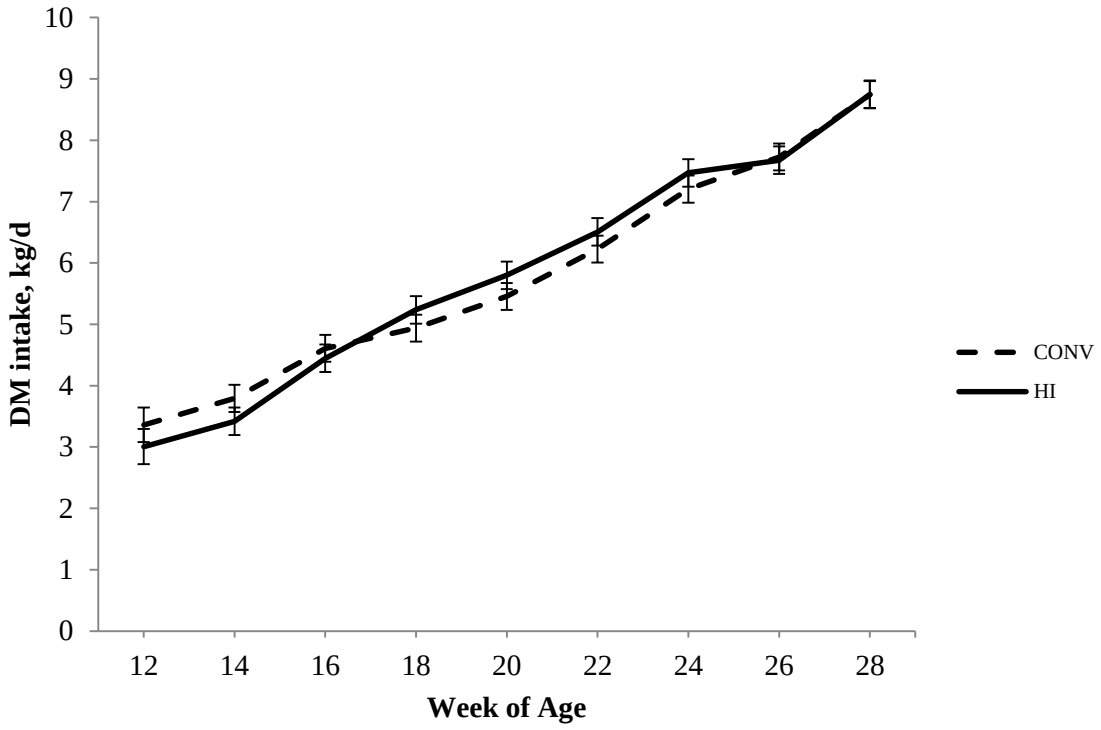

Figure 2.12. Dry matter (DM) intake of weaned calves previously fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. Dry matter intakes were similar overall between pre-weaning treatments ($P = 0.93$).

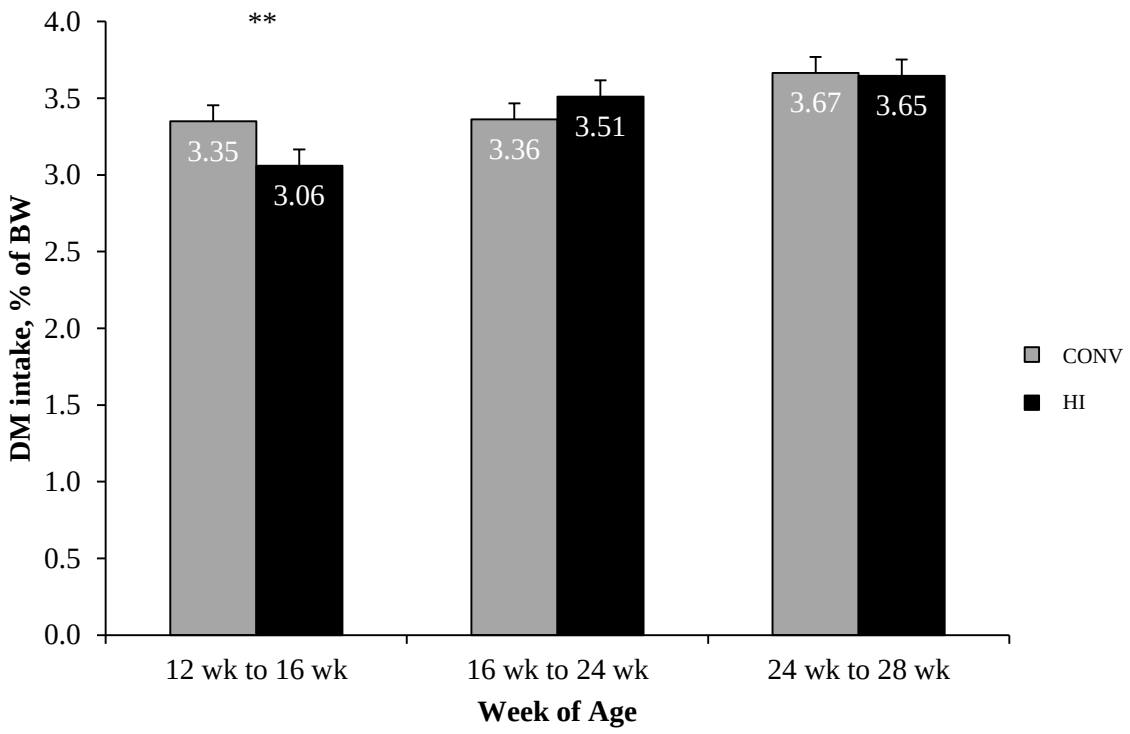

Figure 2.13. Dry matter intake (% of BW) post-weaning for calves previously fed conventional milk replacer (CONV) or high nutrition plane milk replacer (HI). A pre-weaning treatment×time interaction was observed ($P = 0.02$) as calves previously fed CONV consumed more DM from 12 to 16 wk of age compared to calves fed HI ($P = 0.05$); however, DM intakes were similar between pre-weaning treatments from 16 wk of age to the conclusion of the study. $^{**}P \leq 0.05$.

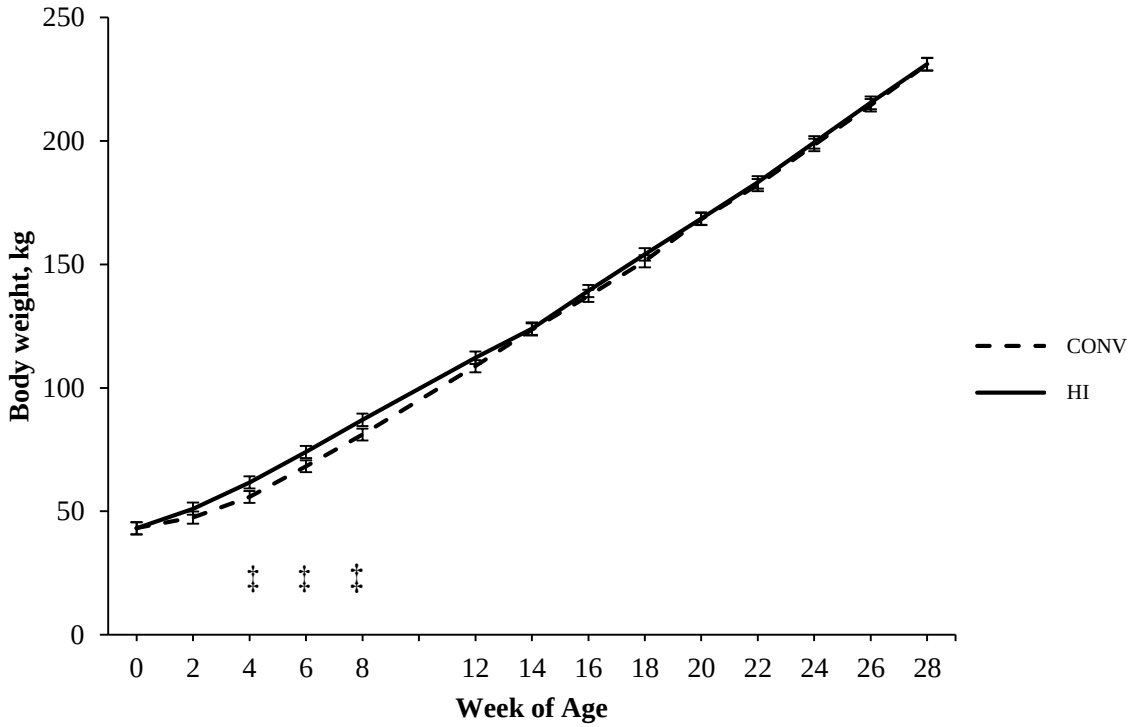

Figure 2.14. Body weight responses from birth through 28 wk of age of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. A treatment×time interaction was observed ($P = 0.02$) as calves fed HI tended to be heavier than calves fed CONV during the pre-weaning period; however, weights began to converge after 8 wk of age and were similar throughout the post-weaning period. ‡$0.10 \leq P < 0.05$.

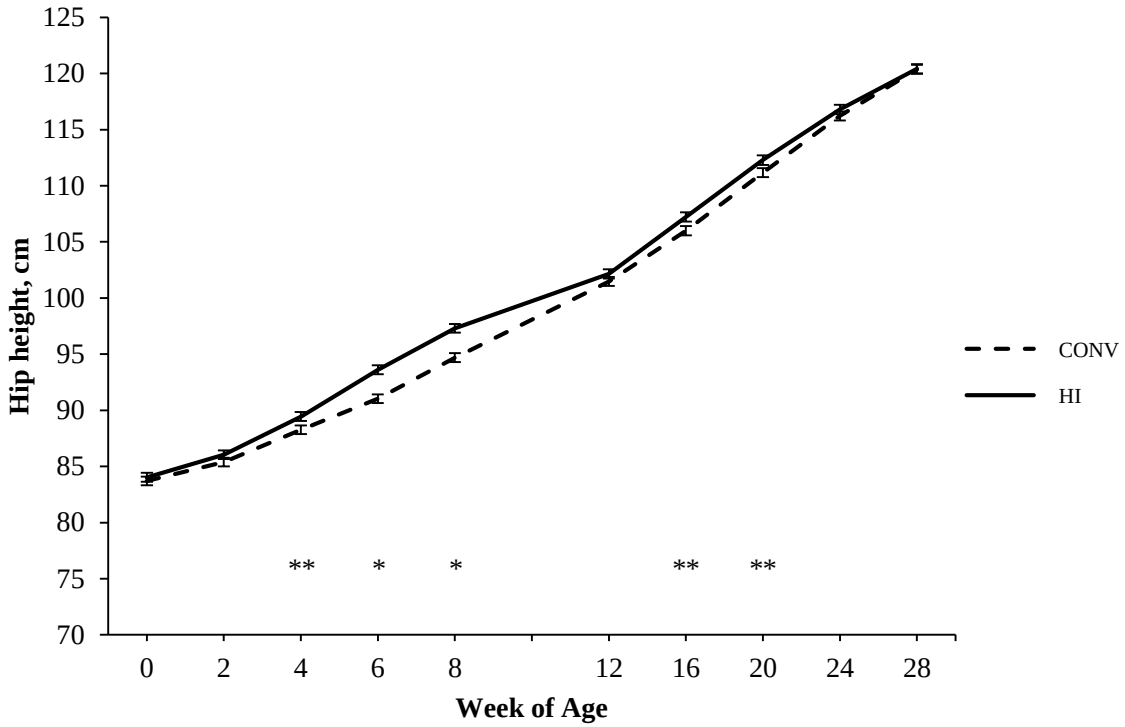

Figure 2.15. Hip height responses from birth through 28 wk of age of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. A treatment×time interaction was observed ($P < 0.01$) as calves fed HI were taller at the hip pre-weaning and early in the post-weaning period, but were similar from 24 to 28 wk of age. **$P \leq 0.05$; *$P < 0.01$.

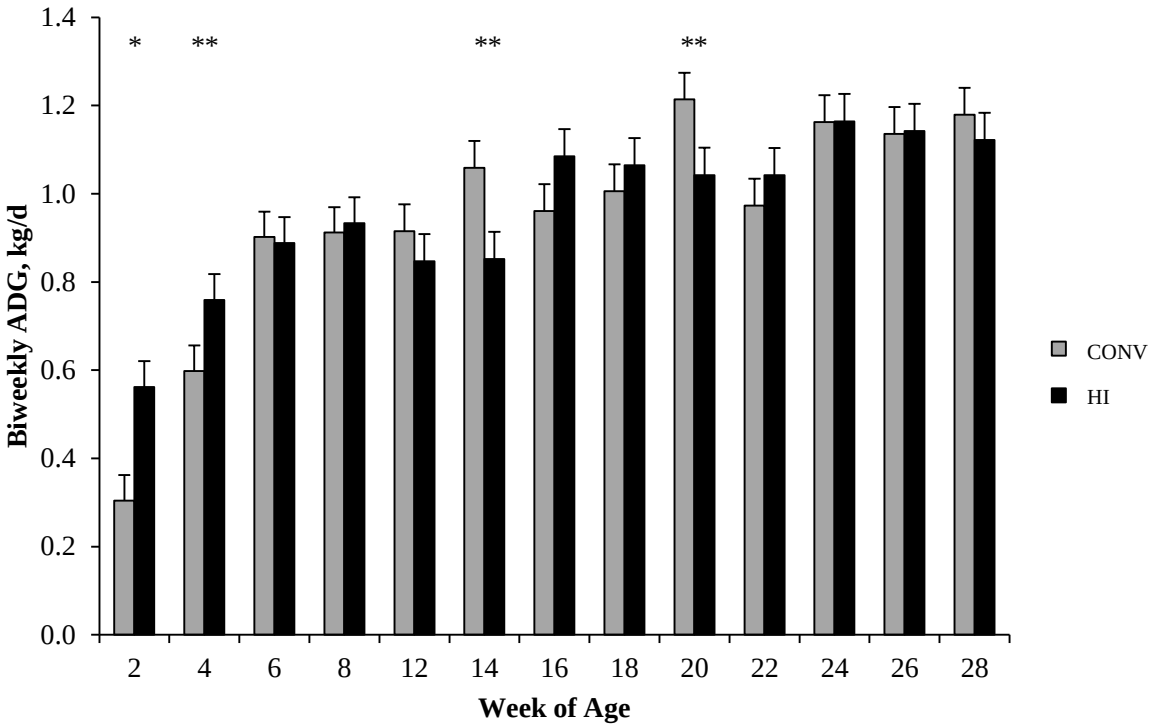

Figure 2.16. Responses in average daily gain (ADG) from birth through 28 wk of age of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning. A treatment×time interaction was observed ($P < 0.01$), as calves fed HI pre-weaning exhibited greater ADG at 2 and 4 wk of age, but ADG was similar through 12 wk of age and was greater for calves previously fed CONV at 14 and 20 wk of age. There was no observed overall effect of treatment ($P = 0.61$). **$P \leq 0.05$; *$P < 0.01$.

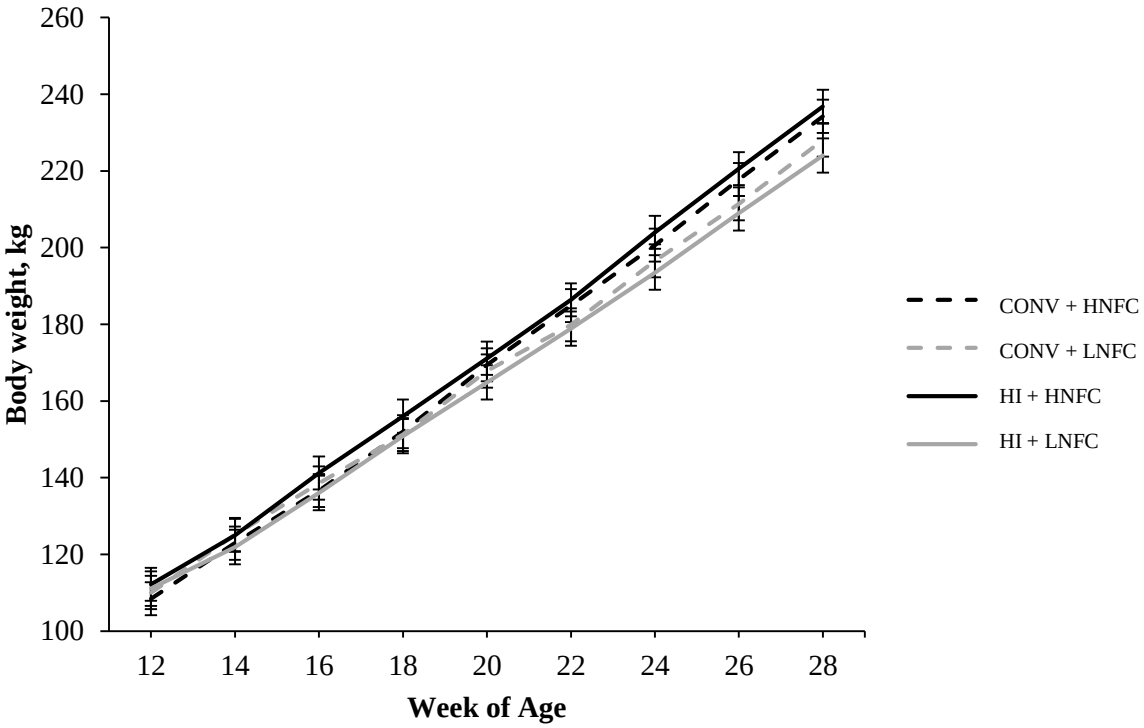

Figure 2.17. Body weight growth curve of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning and low NFC (LNFC) or high NFC (HNFC) post-weaning diets. A pre×post×time interaction ($P < 0.01$) was observed as weights were similar at the beginning of the post-weaning treatment period but diverge starting at 24 wk ($P = 0.09$) for HI+HNFC compared to HI+LNFC. Calves fed HI+LNFC were the lightest at 28 wk of age compared with HI+HNFC ($P = 0.04$) and CONV+HNFC ($P = 0.10$). No pre×post interaction or differences in main effects were observed overall.

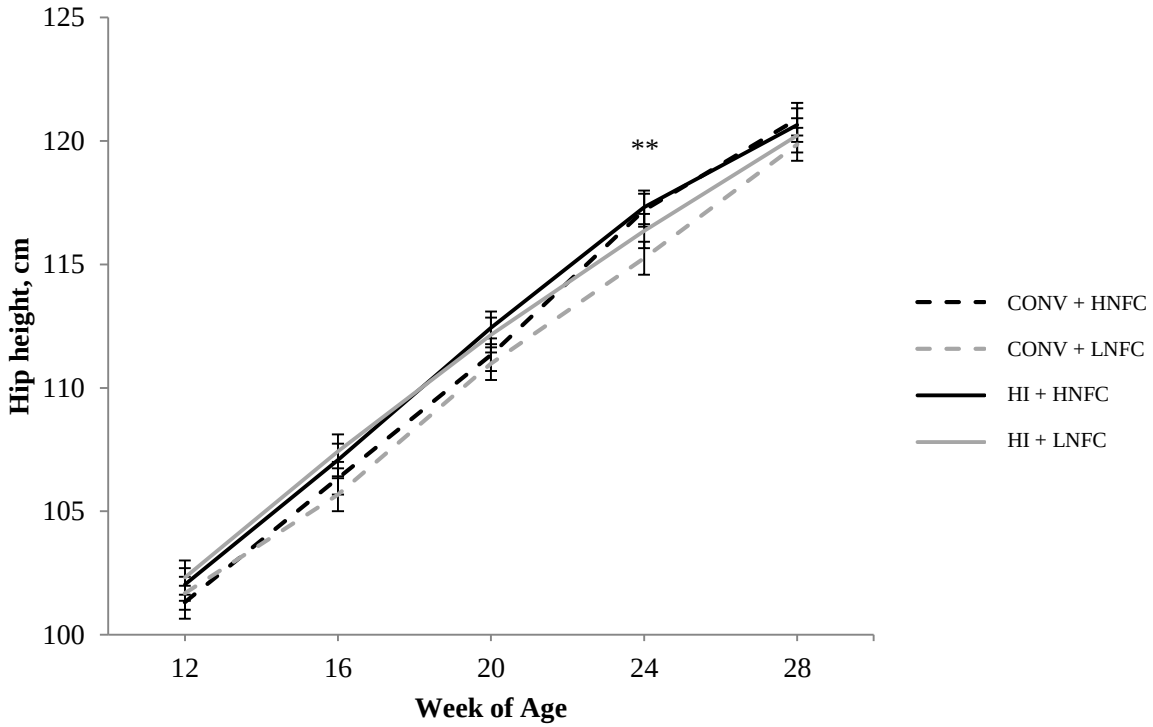

Figure 2.18. Hip height growth curve of dairy calves fed conventional (CONV) or high (HI) planes of nutrition pre-weaning and low NFC (LNFC) or high NFC (HNFC) post-weaning diets. A pre×post×time interaction was observed ($P < 0.01$) as hip height for calves fed HI+HNFC and CONV+HNFC were greater than calves fed CONV+LNFC at 24 wk of age ($P < 0.05$), but were similar among all treatments at 28 wk of age. No pre×post interaction or differences of main effects were observed. *$P \leq 0.05$.

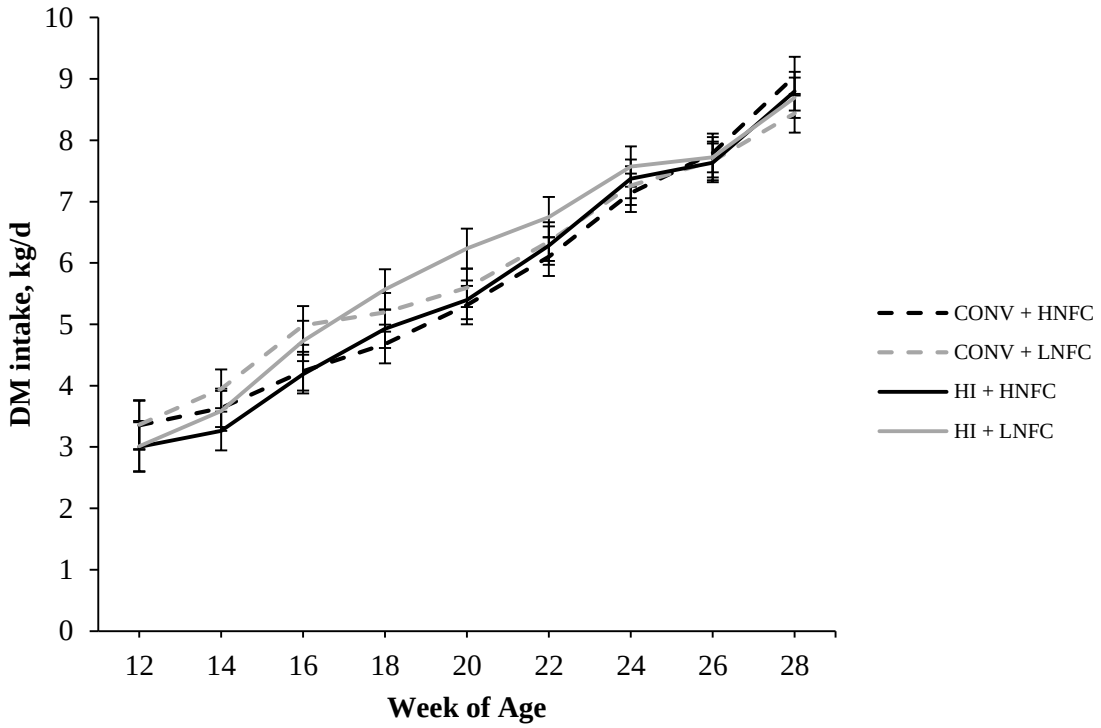

Figure 2.19. Dry matter (DM) intake response of dairy calves previously fed conventional (CONV) or high (HI) planes of nutrition pre-weaning and low NFC (LNFC) or high NFC (HNFC) post-weaning diets from 12 to 28 wk of age. A pre×post×time interaction was observed ($P < 0.01$), as calves fed HI+LNFC consumed more DM than calves fed CONV+HNFC at 18 ($P = 0.05$) and 20 wk ($P = 0.04$) of age, but all treatments were similar from 22 to 28 wk of age.

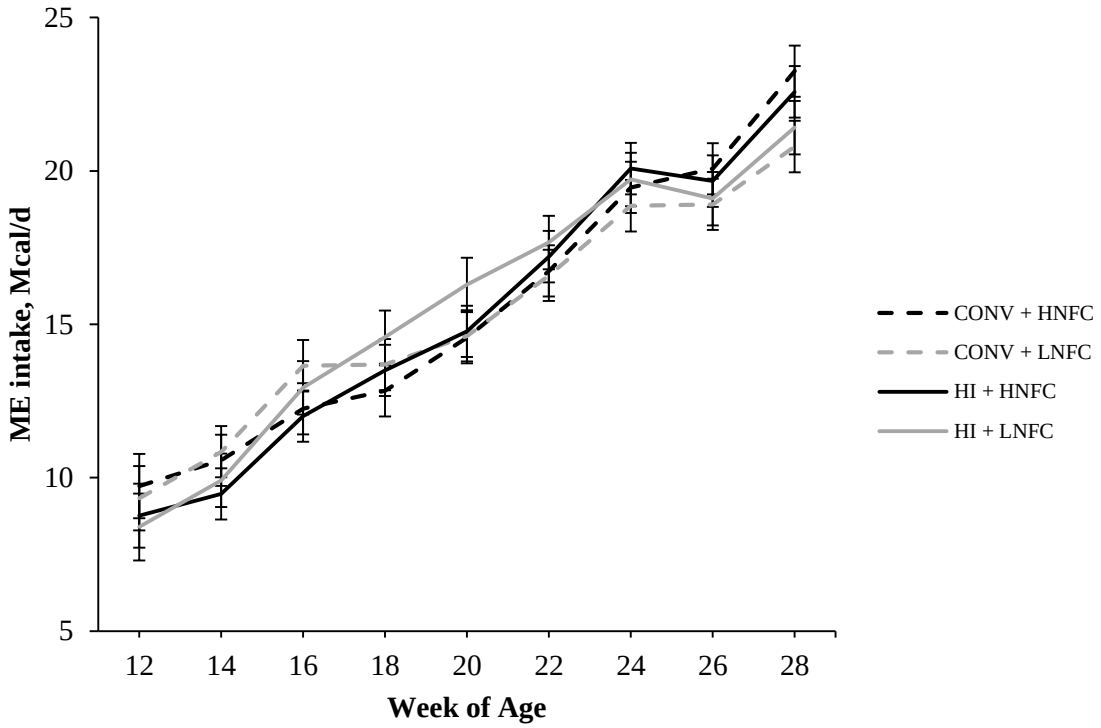

Figure 2.20. Metabolizable energy (ME) intakes of dairy calves previously fed conventional (CONV) or high (HI) planes of nutrition pre-weaning and low NFC (LNFC) or high NFC (HNFC) post-weaning diets from 12 to 28 wk of age. A pre×post×time interaction was observed ($P < 0.01$), as calves fed HI+LNFC consumed numerically more ME than calves fed CONV+HNFC from 18 to 20 wk of age ($P = 0.15$); however, ME intake was numerically greatest for calves fed CONV+HNFC at 28 wk of age.

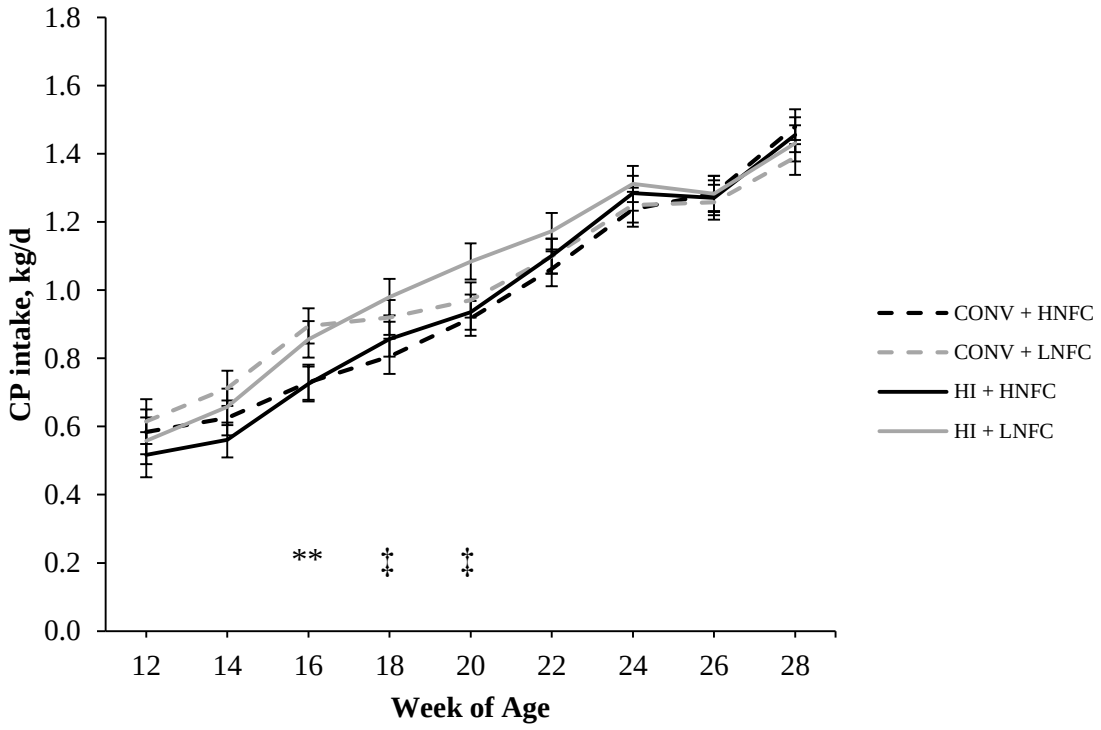

Figure 2.21. Crude protein (CP) intakes of dairy calves previously fed conventional (CONV) or high (HI) planes of nutrition pre-weaning and low NFC (LNFC) or high NFC (HNFC) post-weaning diets from 12 to 28 wk of age. A pre×post×time interaction was observed ($P = 0.01$), as calves fed HI+LNFC consumed more CP than calves fed CONV+HNFC at 16 ($P = 0.09$), 18 ($P = 0.02$), and 20 wk ($P = 0.02$) of age, but all treatments converged from 22 to 28 wk of age. ‡$0.10 \leq P < 0.05$; **$P \leq 0.05$.

CHAPTER 3. INFLUENCE OF DIETARY CARBOHYDRATE FRACTIONS ON GROWTH AND DEVELOPMENT OF PREPUBERTAL DAIRY HEIFERS

3.1 Abstract

Altering dietary carbohydrates and energy in growing dairy heifer diets can potentially influence growth and rumen parameters. The objective of this study was to evaluate the effects of altering dietary non-fiber carbohydrates (NFC) on heifer growth, dry matter intake (DMI), feed efficiency, and blood and rumen metabolites. Ninety Holstein heifers (145.3 ± 25.4 kg, 144 ± 25 d of age) were randomly allocated by body weight (BW) to 1 of 15 pens. Pens were randomly assigned to dietary treatments of high NFC (HNFC; 39% of diet DM), low NFC (LNFC; 30% of diet DM) and low NFC plus fat (LNFC+; 28% of diet DM). Diets were formulated to be isonitrogenous, with lower calculated ME for LNFC compared with HNFC and LNFC+. Heifers were fed diets as a TMR for 112 d, and forage:concentrate ratios were increased from 35:65 to 60:40 on d 57 of the study. Body weights were taken every 2 wk, and hip and withers heights, body condition score (BCS), heart girth, hip width, and blood samples were collected monthly. Rumen fluid was collected esophageally 6 h after feeding from 2 heifers per pen (10 heifers/treatment) to determine pH, NH3, and volatile fatty acids (VFA) monthly. Feeding LNFC+ resulted in heifers that were 4.8 kg and 8.8 kg heavier at the end of the

study compared with HNFC and LNFC, respectively. Average daily gains and feed efficiency were greatest for LNFC+ from d 0 to 56; however, no treatment differences were observed from d 57 to 112. Intake as a percent of BW was greatest for HNFC (3.3%) compared with LNFC (3.1%) and LNFC+ (3.1%) throughout the study. Heifers fed LNFC+ were taller at the hip and withers than heifers fed HNFC and LNFC on d 112. Additionally, LNFC+ resulted in greater BCS compared to LNFC, but not HNFC on d 112. Rumen pH was lower for HNFC from d 0 to 56, but similar among treatments at d 84 and d 112. Proportions of acetate and butyrate were least and greatest, respectively, for HNFC from d 57 to 112. Unexpectedly, increasing dietary NFC did not improve growth compared to a lower NFC diet with added fat despite increased DMI, indicating that energy availability and source may have greater impacts on growth than dietary carbohydrates.

3.2 Introduction

Nutrient requirements of growing dairy heifers have received more attention over the last decade as the industry has recognized that sustainable dairy production relies on well-developed heifers. Several reviews have identified multiple factors related to heifer nutrition that impact the potential for future milk production, including pre-weaning growth rates (Soberon and Van Amburgh, 2013) and DMI at weaning (Heinrichs and Heinrichs, 2011). Increasing growth rates and feed intake usually increases feed costs, which are the largest cost of production for both lactating cows and heifer development (Heinrichs et al., 2013). Strategies to improve feed efficiency and reduce heifer rearing

costs warrant further exploration, as data is limited for growing heifers post-weaning to puberty.

Dietary carbohydrates contribute the largest proportion of energy-yielding products in ruminant diets, and usually are included at rates greater than 70% of the diet for dairy cattle (Nocek and Russell, 1988). Forages are often viewed as inexpensive sources of energy for ruminants; however, per Mcal of ME, starches, sugars, and fats are less expensive to feed as net utilization of fiber is energetically less favorable (VandeHaar and St-Pierre, 2006). Growing heifers are typically fed high-forage diets, which often results in reduced feed efficiency due to reduced digestibility of the forage fraction. Replacing forages with highly digestible concentrates has been shown to increase feed efficiency (Zanton and Heinrichs, 2007) and OM and N digestibility (Zanton and Heinrichs, 2009) when dairy heifers are precision-fed. Concentrate sources provide energy in the form of non-fiber carbohydrates (NFC) in the diet, which includes organic acids, sugars, starches, and neutral-detergent soluble fiber. Different fractions of NFC affect rumen fermentation in different ways, and tend to influence rumen pH and microbial efficiency (Hall and Eastridge, 2014). Carbohydrate type can profoundly affect rumen fermentation, as neutral detergent fiber (NDF) and soluble fiber are predominately metabolized to form acetate, starches to propionate, and sugars to butyrate (Wolin, 1974; Russell and Strobel, 1993). Altering rumen fermentation can have significant impacts on efficiency, as increased acetate:propionate ratios (A:P) have been associated with reduced metabolic efficiency and is typically observed in high-fiber and high-forage diets (Zanton and Heinrichs, 2009).

Rumen development in the neonatal calf is initiated when solid or liquid feeds are introduced into the naïve reticulorumen and fermentation is established. Physical and metabolic development of the rumen is highly dependent on the presence of butyrate and propionate from the fermentation of solid feed (Baldwin et al., 2004). Manipulating rumen fermentation in favor of end products that promote rumen development can potentially improve growth and efficiency of prepubertal dairy heifers. Increased concentrate feeding generally results in increased concentrations of propionate and butyrate, of which 30% to 70% and up to 80% to 90% of each volatile fatty acid (VFA), respectively, is utilized by the rumen epithelium as an energy substrate (Rémond et al., 2007). Though rumen development is well-characterized for the pre-weaned dairy calf, information is limited for development post-weaning. The reticulorumen increases in volume from 30% to nearly 70% of the total foregut volume from birth to weaning (Warner et al., 1956), yet weaned calves are inefficient at utilizing feeds generally considered appropriate for a mature ruminant, namely forages and high-fiber concentrates. McLeod and Baldwin (2000) illustrated an increase in rumen and intestinal mass when ME intake was increased using a high concentrate diet compared to a high forage diet in weaned lambs. It stands to reason that following weaning, there is some capacity for continued rumen development in response to increased ME intake from highly digestible carbohydrates. Therefore, the objectives of this study were to evaluate the effects of dietary NFC on prepubertal dairy heifer growth, FE and rumen fermentation characteristics. Additionally, we wanted to evaluate the effect of ME source on growth to determine if ME from carbohydrate resulted in similar performance to ME from animal fat sources.

3.3 Materials and Methods

3.3.1 Animals and Housing

This study was conducted at the Southern Indiana Purdue Agricultural Center (SIPAC) in Dubois, IN from May 21st to September 10th 2013 using Holstein heifers sourced from Buckeye Heifer Resources of Camden, OH. All heifers were acclimated to facilities and a common diet consisting of a concentrate mix and alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) hay offered in a 35:65 forage:concentrate (F:C) ratio 14 d prior to initiating the study. All animal-related procedures were conducted in compliance with approved protocols from the Purdue Animal Care and Use Committee (PACUC no. 1304000843). Ninety Holstein heifers (145.3 ± 25.4 kg, 144 ± 25 d of age) were weighed on 2 consecutive d at the beginning of the study and assigned by weight to 1 of 15 pens with 6 heifers per pen. Housing consisted of a naturally ventilated barn with 3.7 m x 21.9 m pens, 3.7 m of feed bunk space, and unrestricted access to water. Pens were covered mid-way by slanted steel roofing and bedded with sawdust throughout the study as needed. On d 0, heifers were given magnet boluses, dewormed (Dectomax pour-on; Zoetis, Florham Park, NJ), and vaccinated for bovine viral diarrhea, infectious bovine rhinotracheitis, and leptospirosis (Bovi-Shield Gold FP5 L5 HB; Zoetis) and 7 strains of *Clostridium* (Ultrabac 7; Zoetis) and were boostered 4 wk later.

3.3.2 Experimental Design and Treatments

Pens were randomly assigned to treatment diets containing high NFC (HNFC), low NFC (LNFC), or low NFC with added fat (LNFC+) concentrate mixes with the remainder of the diet offered as chopped dry hay. Dietary F:C on a DM basis were 35:65 from d 0 to 56 and 60:40 from d 57 to 112. Feed was delivered with the grain mix top-dressing hay and was offered once per d at 0700 h throughout the study. Ingredient and nutrient composition of grain mixes and forages used in this study are presented in Table 3.1. Diets were formulated according to NRC (2001) recommendations to allow 0.90 kg/d of ADG for growing Holstein heifers. Feed was initially offered at approximately 2.8% of the average pen BW and was adjusted daily to allow for *ad libitum* intake and minimize refusals (<10% daily). Hay used for the treatment diets was harvested at SIPAC in 2012 from a second cutting of an alfalfa/orchardgrass mixture and for the common diet was an alfalfa/orchardgrass purchased off-site. Orts were weighed and sub-sampled once per wk to determine weekly pen intakes. Feed ingredients and orts were dried at 60°C in a forced air oven, ground through a 1.0 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ), composited by month, and analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Labs, Ithaca, NY). Samples were analyzed for CP (AOAC 984.13, AOAC, 1990), NDF (Van Soest et al., 1991), ADF (AOAC 973.18, AOAC, 1990), ME (calculated from TDN in feed; NRC, 2001), and minerals (inductively coupled plasma spectrometry; Isaac and Johnson, 1985).

3.3.3 Data Collection and Analysis

Heifers were weighed every 2 wk during the study and skeletal growth measurements, including withers height (WH), hip height (HH), heart girth circumference (HGC), and hip width (HW) were assessed monthly. Body condition score (BCS) was determined monthly on a scale of 1 to 5 (1 = emaciated, 5 = obese; Edmonson et al., 1989) by 2 evaluators and averaged. Blood samples (10 mL) were collected via jugular venipuncture monthly into evacuated blood tubes containing lithium heparin (BD Diagnostics, Franklin Lakes, NJ). Plasma was aspirated following centrifugation (2500 x *g* for 15 min at 4°C) and frozen at -20°C for later analysis. Plasma was analyzed for plasma urea N (PUN; procedure no. 0580; Stanbio Laboratory Inc., San Antonio, TX) and glucose (procedure no. 1070; Stanbio Laboratory Inc., San Antonio, TX). Rumen fluid was obtained as described by Dennis et al. (2012) on d 0, 28, 56, 84, and 112 using an esophageal tube from 2 heifers in each pen and analyzed for pH, VFA, and rumen NH3. Rumen fluid pH was immediately determined (model EL2; Mettler-Toledo, Columbus, OH), and two 10 mL samples of fluid were acidified using 25% w/v meta-phosphoric acid (4:1 sample:acid ratio) and frozen at -20°C for later analysis. Rumen fluid samples were analyzed for VFA using gas chromatography on a bonded capillary column (Supelco, Bellefonte, PA; Erwin et al., 1961) and for NH3 using the Kjeldahl procedure (FOSS Kjeltec 2300, Hoganas, Sweden; AOAC 984.13, AOAC, 1990).

3.3.4 Statistical Analysis

Data were analyzed overall and by period to determine treatment and carryover effects into the grower period. Pens were assigned to treatments in a completely randomized design, with heifers randomly allocated to pens by BW to obtain a similar average BW in each pen. Growth and intake data were analyzed as repeated measures (Littell et al., 1998) using the MIXED procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Treatment, time, and the interaction of the two variables were included in statistical models as fixed effects and starting measurements were included as covariates where appropriate. Pen nested within treatment was considered random for growth, intake, blood metabolites, rumen pH, VFA, and NH3 models. Variance-covariance matrix structures were evaluated for each model using simple, first order auto-regressive, compound symmetry, and unstructured covariance structures and were selected for each model based on the lowest Bayesian information criterion fit statistic. Least squares means and standard errors of the mean are reported on a per heifer basis and mean differences were separated using the Tukey-Kramer method. When interactions of fixed effects were significant, the SLICE option was used to determine the treatment significance at the various time points. Pearson correlation coefficients were determined using the CORR procedure to identify relationships between DMI and chemical composition of the diets over the entire study. Statistical differences were considered significant at $P \leq 0.05$ and trends at $0.10 \geq P > 0.05$.

3.4 Results and Discussion

3.4.1 Heifer Weight and Skeletal Growth

Body weights, ADG, and skeletal growth responses to changes in dietary NFC are outlined in Table 3.2. Heifers fed LNFC+ were heavier on d 56 ($P < 0.01$) and d 112 ($P < 0.01$) of the study compared to heifers fed LNFC. Heifers on the HNFC diet were intermediate and tended to be lighter on d 56 ($P = 0.09$) and d 112 ($P = 0.07$) compared to heifers fed LNFC+. However, treatment effects were not detected for overall BW gain from d 0 to d 112 ($P = 0.13$) despite weight advantages for heifers fed LNFC+ at the conclusion of the study. When analyzed by study period, overall effects of treatment on BW gain were mainly apparent when F:C in the diet was lower (d 0 to 56). When diets were adjusted to a 60:40 from a 35:65 F:C, BW only tended to differ ($P = 0.10$) among treatments, though heifers fed LNFC+ were 7.1 kg heavier on average from d 57 to 112 than heifers fed LNFC ($P = 0.04$). Average daily gain in the lower F:C feeding period was 14.9% and 8.9% greater for heifers fed LNFC+ compared to heifers fed LNFC ($P < 0.01$) or HNFC ($P = 0.05$), respectively. Following a diet adjustment, however, ADG was similar among treatments ($P = 0.86$), resulting in no overall effect of dietary NFC on ADG ($P = 0.13$). Several studies have illustrated increased growth rates with increasing energy concentration for growing beef (Houseknecht et al., 1988; Hall et al., 1995; Yelich et al., 1995) and dairy heifers (Radcliff et al., 1997; Davis Rincker et al., 2008b), all of which indicated an increase in body adiposity with increasing energy intake. In contrast, Amos (1986) reported improved ADG when low energy compared to high energy diets were fed to 4 mo old Holstein heifers and steers. However, DMI were reduced for calves fed high energy diets, which the authors attributed to inclusion of beef

tallow at rates greater than 5% of the diet DM (Amos, 1986). Other studies have also observed reduced DMI with increasing fat inclusion in the diets of weaned lambs (Seabrook et al., 2011), feedlot cattle (Zinn, 1989; Zinn et al., 1994), and lactating cows (Choi and Palmquist, 1996; Relling and Reynolds, 2007), particularly when in excess of 5% of the diet DM. However, DMI was not depressed in the current study for heifers fed LNFC+ (intake discussion below). Rates of gain observed in the current study agree with those observed by Bethard et al. (1997), as prepubertal Holstein heifers fed high energy diets based on corn silage and orchardgrass hay exhibited 31% greater ADG compared to heifers fed low energy diets. Dietary fiber likely influenced performance in the study from Bethard et al. (1997), as ADF content averaged 33.6% for low energy diets and 25.9% for high energy diets, which resulted in a 26% increase in DMI for heifers fed high energy diets. Growth rates were also similar to those observed by Anderson et al. (2015) for prepubertal heifers (initially 133 d of age) limit-fed to 2.45% of BW on DM basis diets with low- or high-fat DDGS with equal ME content. Diets ranged from 20.6 to 32.1% NFC and ADG averaged 0.96 kg/d (Anderson et al., 2015). Differences in energy digestibility may explain growth rate responses in the higher concentrate feeding period as dietary fats and starches are more readily utilized than dietary fiber; however, digestibility coefficients were not determined.

Frame growth exhibited similar responses to those observed for BW and ADG (Figures 3.2 and 3.3). Overall, heifers fed LNFC+ were the tallest for HH ($P < 0.01$) and WH ($P < 0.01$) on d 56 and d 112 compared to heifers fed LNFC. Heifers fed HNFC were intermediate for HH and WH, but tended to be shorter at the hip ($P = 0.10$) and withers ($P = 0.10$) than heifers fed LNFC+ on d 56 and significantly shorter on d 112 (*P*

< 0.01). Heifers fed LNFC (P = 0.04) and LNFC+ (P = 0.05) were wider at the hips than heifers fed HNFC on d 56, but not on d 112. However, HW and HGC were similar among treatments at the end of the study. Monthly gain in HH (P = 0.10) and WH (P = 0.07) tended to differ in favor of heifers fed LNFC+ compared to heifers fed LNFC, with HNFC-fed heifers having intermediate growth (Table 3.2); however, total growth of all other skeletal measurements were similar among treatments over time. Davis Rincker et al. (2008) also observed increased WH and HW, in addition to increased ADG, when high-energy diets were fed for 6 or 12 wk compared with 0 or 3 wk. However, Whitlock et al. (2002) evaluated diets with increasing CP:ME (48.1, 56.8, and 66.0 g of CP/Mcal of ME, respectively) fed for 1.2 kg/d of ADG and reported similar ADG and WH across treatments. Anderson et al. (2015) also reported ADG, HH, WH, and HGC were similar for prepubertal heifers fed a high-fat DDGS diet (20.6% NFC) compared to a high NFC (32.1% NFC) control diet with equal energy intakes. However, Anderson et al. (2015) fed diets with approximately 5% lower ME content and 13% higher NDF content compared to diets fed in the current study which may partially explain lack of response to energy source. Our data suggests that energy availability plays a larger role in optimizing growth for prepubertal heifers.

Heifers fed HNFC had significantly higher BCS compared to heifers fed LNFC (P = 0.01) at the conclusion of the study (Table 3.2), with heifers fed LNFC+ intermediate and tending to have greater BCS than heifers fed LNFC (P = 0.06). Heifers fed LNFC+ carried more body condition than heifers fed LNFC throughout the study (P < 0.01), and carried similar body condition to heifers fed HNFC, except for on d 56 of the study when heifers fed HNFC exhibited lower BCS compared to LNFC+ (P = 0.05). However,

absolute differences in BCS between treatments were less than 0.2 units on d 56 and 112, which is below a realistic threshold of measurable and biological difference in condition. Tikofsky et al. (2001) fed increasing levels of fat at equal energy intakes to pre-weaned Holstein bulls and observed increased FE due to a trend in increased apparent partial efficiency of energy intake use and increased carcass energy retention. Protein retention was similar among treatment groups, leading the authors to conclude that lower fat diets resulted in larger fractions of available energy being utilized for protein deposition whereas higher fat diets used ME from fat to fuel protein deposition with excess energy deposited as adipose tissue (Tikofsky et al., 2001). In a similar way, Garrett (1980) recognized that cattle fed high levels of concentrates post-weaning would likely exhibit more body fat at a similar weight than those fed lower energy diets, as was the case for heifers fed HNFC compared to LNFC in the current study. As skeletal growth was increased over time for heifers fed LNFC+ despite similar ME intake compared to HNFC, it is likely that available energy from fat was more efficiently utilized for structural growth compared to starch and other NFC sources predominately provided in the HNFC diet.

3.4.2 Dry Matter and Nutrient Intake

Intake responses to altered dietary carbohydrate composition are presented in Table 3.3. Average daily DMI differed over time, though DMI was similar from d 0 to 56 ($P = 0.45$), but greatest for heifers fed HNFC on d 84 ($P < 0.01$), d 98 ($P < 0.01$), and d 112 ($P = 0.03$) compared to heifers fed LNFC diets. As a percent of BW, DMI was greatest for heifers fed HNFC throughout the study, though most of the dietary effect was

observed from d 56 to the end of the study (Figure 3.4). Following a diet change, DMI (percent of BW) declined 9.7, 12.0, and 13.2% from d 56 to 70 for LNFC, HNFC, and LNFC+, respectively. Heifers fed LNFC+ had the lowest intake as a percent of BW on d 70 ($P < 0.01$), which potentially could be related to a negative-associative effect of increased fat intake and increased forage inclusion. Park et al. (1983) increased dietary fat from 3.6 to 13.1% of the diet on a DM basis using sunflower seeds and observed reduced DMI with increasing concentrations of plant fats. Similarly, Zinn and Plascencia (1996) observed that DMI tended to decrease when supplemental animal fat was given to growing feedlot steers fed either a 10 or 30% alfalfa hay diet. While the mechanism by which fat suppresses DMI in cattle remains poorly understood, it is thought that reduced fiber digestibility in the rumen, as well as signaling of gut hormones responsible for satiety, may play a role (Allen, 2000). In the current study, dietary fat ranged from 4.6 to 6.1% throughout the study for heifers fed LNFC+ and may not have been high enough to consistently depress intake in heifers fed LNFC+ compared to LNFC. Though the reason for a lack of significant differences in intake during the first 56 d of the current study are unclear, it is likely that increased concentrations of NDF in LNFC and LNFC+ physically restricted intake when more forage was included in the diet from d 57 to d 112, as well as potentially reduced digestibility, resulting in increased retention time in the rumen of LNFC diets compared to heifers fed HNFC. Voluntary intake in dairy cattle is influenced by both physical and chemical factors (Allen, 2000), and in the current study, DMI was negatively correlated with dietary NDF only during the grower period ($r = -0.35$; $P < 0.01$). Classical studies evaluating the relationship between voluntary intake and detergent fiber fractions have also illustrated negative correlations of dietary NDF with

DMI (Van Soest, 1965), though with a much stronger relationship than that observed in the current study. Possible explanations for discrepancies between older literature and the current study are that previous studies focused on evaluating voluntary intake have predominately evaluated all-forage diets (Jung and Allen, 1995), and negative-associative effects of feeds used in the current study may have contributed to the variation in intake response potentially not attributed to NDF.

Intake of dietary NDF was significantly greater for heifers fed LNFC diets compared to HNFC during the earlier low F:C feeding period ($P < 0.01$) and overall ($P < 0.01$), as designed. However, NDF intake was similar among treatments during the grower period due to increased total DMI for heifers consuming HNFC compared to heifers fed LNFC diets. Similarly, NDF intake as a percent of BW was greatest for heifers fed LNFC diets during the lower F:C feeding period and overall. A treatment×time interaction was observed during the grower period ($P < 0.01$), as NDF intake as a percent of BW was greatest for heifers fed LNFC from d 57 to 70 but similar among treatments from d 71 to 84 and d 85 to 98 (Figure 3.5). Forage NDF intake was greatest for heifers fed LNFC+ and LNFC compared with HNFC overall, averaging 1.43, 1.41, and 1.29 kg/d, respectively. Additionally, a treatment×time interaction was observed for fNDF intake as a percent of BW (Figure 3.6). Heifers fed LNFC and LNFC+ consistently had greater intake of fNDF compared to HNFC from d 0 to d 56; however, following a diet adjustment to higher forage inclusion, heifers fed LNFC consumed more fNDF from d 57 to d 70 compared to LNFC+ ($P < 0.01$) and HNFC ($P < 0.01$), and heifers fed LNFC+ consumed more fNDF than heifers fed HNFC ($P = 0.02$). In contrast to NDF intake, NFC and starch intake overall was, on average, 1.3 ($P < 0.01$)

and 1.8 ($P < 0.01$) times greater for heifers fed HNFC compared to LNFC and LNFC+ diets, respectively. Differences in carbohydrate intake were designed to differ among treatments; however, responses in total DMI during the higher concentrate feeding period were unexpected. As total NDF content of LNFC diets exceeded 40% in the higher concentrate feeding period, DMI was expected to be depressed compared to feeding a HNFC diet. Inclusion of soybean and cottonseed hulls in all the grain mixes, and wheat middlings in the LNFC grain mixes, may have increased passage rate more than anticipated in the current study, resulting in similar DMI from d 0 to d 56 and NDF intakes from d 56 to d 112. Grant (1997) proposed a simple model for interactions among forage level and non-forage fiber sources and indicated that lower forage diets have less potential for entrapment of small particles, resulting in greater passage rate of non-forage fiber sources and less potential for ruminal digestion. Inclusion of cottonseed hulls in calf starters for Holstein calves up to 15% of starter DM increased starter intake in calves fed whole milk (Hill et al., 2009a). Similarly, diets including cottonseed hulls at 7.8% of dietary DM for early lactation cows increased DMI approximately 8% over diets without cottonseed hulls (Kononoff and Heinrichs, 2003). Other non-forage fiber sources, such as soybean hulls and wheat middlings, have also been shown to increase DMI and passage rate in lactating dairy cattle (Firkins, 1997; Grant, 1997). Inclusion of 33.7 to 36.5% non-forage fiber sources for LNFC and LNFC+ diets may partially explain similar DMI to HNFC observed during the lower F:C feeding period (d 0 to 56). Forage NDF and non-forage fiber sources can differ considerably in rumen fermentability and total tract NDF digestibility. When comparing corn gluten feed and soybean hulls as sources for partial replacement of dietary NDF in corn silage/alfalfa hay diets, Sarwar et

al. (1991) observed improved apparent total tract NDF digestion when fNDF was reduced from 85% to between 45% and 65% of total dietary NDF. Partial explanation for this response was a reduction in OM intake for diets with partial replacement of fNDF with non-forage fiber sources (Sarwar et al., 1991). As non-forage fiber sources tend to exhibit equal to faster rates of passage and equal to slower NDF degradation rates compared to forages (Firkins, 1997), forage NDF likely plays a larger role in regulating intake than total NDF.

Intakes observed in the current study disagree with those reported by Hoffman et al. (2008) for pen-fed Holstein heifers. Dry matter intakes were, on average, 21.9% greater for all heifers during the lower F:C feeding period compared to heifers fed diets similar in CP and NDF content (Hoffman et al., 2008). Additionally, when dietary NDF was increased during the grower period, average DMI were 9.3% greater than those reported by Hoffman et al. (2008) for heifers fed diets with similar NE_m and lower NDF content (45.0% in the current study vs. 38.7%). Those authors did not report diet composition for pen-fed heifers, and reasons for disagreement in DMI between Hoffman et al. (2008) and the current study are unclear. However, it is common to feed growing dairy heifers diets containing corn silage and other ensiled forages, which have been shown to depress DMI compared to diets with higher DM content fed to growing heifers (Thomas, 1961) or mature cows (Lahr et al., 1983). As NE_m for diets in the current study were similar or lower (5.7% lower in the 60:40 F:C feeding period) than those reported by Hoffman et al. (2008), greater intakes observed in the current study may indicate heifers were consuming more feed to meet maintenance energy requirements due to greater total NDF content in the diet.

During the lower F:C feeding period, ME intake was significantly greater for heifers fed LNFC+ compared to LNFC ($P < 0.01$) and tended to be greater compared to heifers fed HNFC ($P = 0.10$). Additionally, CP intake was significantly greater for heifers fed LNFC+ compared to HNFC on d 14 ($P = 0.05$) and d 28 ($P = 0.02$) during the lower F:C feeding period. Increased ME and CP intake for heifers fed LNFC+ likely resulted in increased heights and growth rates compared to heifers consuming HNFC and LNFC. The ratio of CP:ME intake was 58.6, 59.1, and 63.2 g of CP/Mcal of ME for LNFC+, HNFC, and LNFC, respectively, during the first 56 d of the study. Gabler and Heinrichs (2003) evaluated increasing proportions of CP:ME on prepubertal dairy heifer performance and observed linear increases in feed efficiency and trends for linear increases in frame growth rates. These results are in contrast to those outlined in the current study, though heifers in the previously described study were fed for restricted ADG of approximately 0.80 kg/d, whereas heifers in the current study were fed for ad libitum intake and unrestricted ADG. Additionally, average CP intake of heifers fed the lowest CP:ME diet was over 55% lower than levels recommended by the NRC (2001), which the authors attributed to the observed linear responses in FE (Gabler and Heinrichs, 2003). Lammers and Heinrichs (2000) fed diets ranging from 46.1 to 61.1 g CP/Mcal ME to heifers starting at 28 wk of age and found that FE and frame growth increased with increasing CP:ME ratio. Both previously described studies fed diets with varying levels of CP and maintained similar ME concentrations, whereas the current study altered ME by manipulating NFC and NDF concentrations and fat levels in the diet while maintaining similar CP content. Recently, Hill et al. (2013) evaluated literature on CP requirements of heifers since the NRC (2001) was published and suggested that from

4 mo to breeding age, optimal CP:ME ratios range from 61 to 65 g of CP/Mcal of ME. However, it appears from the current study that diet composition will affect appropriate proportions of CP:ME depending on which nutrients are manipulated. Energy from lipids theoretically provides 9 kcal/g of ME, compared to simple and complex carbohydrates theoretically providing 4 kcal/g of ME. As efficiency of ME utilization has been reported between 60 and 80% in ruminants, when growth rates increase post-weaning with energy intakes above maintenance requirements, the rate of protein deposition is maximized and excess energy is deposited as fat (Garrett, 1980). Reynolds et al. (1991) observed that when beef heifers were fed for constant ME intake, whole body heat production was lower and tissue energy retention was greater for heifers fed 75% grain versus 25% grain, illustrating the importance of dietary energy source consideration in growing heifer diets.

3.4.3 Feed and Nutrient Efficiencies

From d 0 to 56, treatment tended to affect feed efficiency (gain-to-feed; G:F), as heifers fed LNFC+ were 12.7% more efficient than heifers fed LNFC and 9.3% more efficient than heifers fed HNFC, with a trend ($P = 0.07$) towards improved feed efficiency for LNFC+ from d 0 to d 112 as compared to HNFC. During the grower period, a tendency for a treatment×time interaction was observed ($P = 0.10$) as heifers fed HNFC were less efficient than heifers fed LNFC ($P = 0.03$) after a diet adjustment to higher forage inclusion, and heifers fed LNFC+ were more efficient than heifers fed HNFC on d 98 ($P = 0.03$). Net efficiency of fiber utilization, whether from forage or non-forage sources, is generally lower than that of starch and fat (VandeHaar and St-

Pierre, 2006), though there were no detectable differences overall between G:F of high and low NFC diets in the current study. However, there was an advantage in G:F when fat was added to the higher fiber diet during first half of the study when heifers were younger. Anderson et al. (2015), however, did not observe an improvement in feed efficiency when diets with 7.0% fat were fed to prepubertal heifers compared to diets with 2.9% fat. Diet adjustment to a higher forage diet resulted in no favorable improvement in G:F for heifers fed LNFC, though HNFC-fed heifers had numerically lower G:F from d 56 to d 112, and significantly lower G:F compared to LNFC only from d 56 to d 70. This suggests a need for gradual changes when making large diet adjustments from high grain to high forage diets for growing heifers when NFC and starch concentrations are high. Interestingly, ME efficiency, expressed as kg of ADG per Mcal of daily ME intake, was not significantly affected by treatment overall ($P = 0.26$) despite differences in dietary ME for LNFC compared to HNFC and LNFC+. However, higher ME content in HNFC and LNFC+ diets supported greater ADG and ME intakes overall compared to LNFC. In contrast, overall CP efficiency, expressed as kg of ADG per kg of daily CP intake, was significantly improved for heifers fed LNFC+ compared to LNFC ($P = 0.04$) and tended to improve compared to heifers fed HNFC ($P = 0.07$). Increased ADG for LNFC+ compared to LNFC partially explains the response in CP efficiency, and supports the theory that protein utilization is optimized only when energy is sufficient for growth as protein deposition is energetically more costly than adipose deposition (Garrett, 1980). It is unclear why LNFC+ tended to be more favorable with respect to CP utilization compared to HNFC, though can be partially explained by increased DMI for HNFC to attain similar ADG to LNFC+ overall. Geay (1984)

reported a curvilinear relationship between the proportions of energy retained as protein and ME efficiency for growth, where greater protein deposition is the result of lower ME efficiency as protein deposition is more energetically costly. In the current study, it appears that the dietary source of ME, whether from fat or starch, may affect the relationship of protein and energy utilization in growing heifers. This could be partly due to differences in energy expenditure for digestion, as the thermic effect of dietary fat is lower than that of starch or fiber, or from the ruminal end products of fermentation, though neither explanation is consistently observed throughout the literature (Garrett, 1980).

3.4.4 Feed Costs

Costs of feeding heifers during the study are reported in Table 3.5. Feeding LNFC to growing heifers resulted in overall cost savings of $0.22 and $0.13 per heifer/d compared to feeding HNFC or LNFC+, respectively ($P < 0.05$). A treatment×time interaction was observed overall ($P < 0.01$), as cost per heifer/d was similar for heifers fed HNFC and LNFC+ on d 14, d 28, and d 42, but higher for heifers fed HNFC thereafter until the conclusion of the study (Figure 3.4). Heifers fed LNFC maintained the lowest cost per heifer/d throughout the study, though costs were similar to heifers fed LNFC+ on d 70 and d 84. Daily feed costs per heifer subsequently increased as DMI increased, and were greatest, on average, on d 56 of the study at $1.84 per heifer/d. Feed costs per kg of ADG were lowest for heifers fed LNFC+ compared to HNFC from d 0 to 56 ($P = 0.03$), resulting in a cost savings of $0.30 per kg of gain. However, feed costs per kg of ADG were similar among treatments overall ($P = 0.37$), averaging $2.17 per kg of

gain. When priced on an energy basis, supplemental fats and concentrates are typically less expensive to feed than high fiber by-products and forages. Increased concentrate consumption, therefore, usually results in increased income over feed costs for lactating cows depending on forage quality provided (Smith, 1976). In our study, a larger proportion of the HNFC diet included corn and DDGS, resulting in greater costs per ton for the grain mix due to higher corn prices from the 2012 crop year. When accounting for average commodity prices from 2008 to 2013 crop years, costs per kg of gain were \$0.42 greater for heifers fed HNFC compared to LNFC+ from d 0 to 56 ($P < 0.01$). Paired with increased DMI for heifers fed HNFC, our data suggests that alternative energy sources, such as supplemental fat, may be more cost-effective when grain prices are high for feeding growing heifers.

3.4.5 Blood Metabolites

Blood glucose was measured in the current study to give insight to energy status of growing heifers in response to dietary carbohydrates (Table 3.5). Overall, blood glucose concentrations were similar among treatments, but exhibited a tendency for a treatment×time interaction ($P = 0.09$). Heifers fed LNFC+ had elevated glucose concentrations compared to HNFC ($P = 0.01$) and LNFC-fed ($P < 0.01$) heifers on d 28, and elevated levels compared to HNFC ($P = 0.04$) on d 84 of the study (Figure 3.7). This result was unexpected as increased starch intake and subsequent fermentation was expected to yield more propionate production, thereby increasing gluconeogenesis and elevating blood glucose. However, as proportions of propionate were similar among treatments, it is possible that the response to fat supplementation was influenced by lower

insulin response, therefore reducing glucose clearance. Bunting et al. (1996) observed an effect of fat level on blood glucose and insulin in 3 mo old Holstein steers, where blood glucose increased 6.1% over control post-prandially for steers fed prilled, hydrogenated tallow and insulin concentrations declined 39.5% compared to control for increased fat concentrations in the diet. Schoonmaker et al. (2003) reported elevated post-prandial serum insulin for steers fed an all-concentrate compared to an all-fiber diet, suggesting higher glucose utilization by peripheral tissues in response to greater glucose supply from a high concentrate diet as circulating glucose concentrations were similar between diets. As heifers fed LNFC+ increased in body condition from d 0 to d 56, it may be that increased adiposity influenced insulin sensitivity early in the study, thereby reducing glucose clearance rate. As heifers fed HNFC increased in body condition, glucose responses were similar on d 84 to those observed for heifers fed LNFC+. Dietary fat is known to reduce glucose uptake and oxidation in response to increased adipose tissue deposition, thereby reducing insulin sensitivity (Chilliard, 1993). However, circulating insulin was not measured in this study to give insight to hormonal responses to increased starch and fat intake. Additionally, changes in BCS were slight and below a biologically measurable level, therefore body fat content may play a minor role in glucose metabolism in this study.

Plasma urea N concentrations were similar among treatments throughout the study ($P = 0.79$), which agrees with observed CP intakes which were similar overall ($P = 0.49$). A treatment×time interaction was observed for CP intake, as heifers fed HNFC consumed more CP per d from d 84 to d 112 ($P < 0.05$) due to increased overall DMI during this period. However, this did not translate into elevated PUN concentrations

during the grower period, which may indicate an improvement in N utilization for heifers fed HNFC diets with a greater F:C ratio. Concentrations ranged from 8.2 to 12.1 mg/dL of urea N across all treatments during the study, which has been reported to illustrate optimal N utilization in growing cattle (Byers and Moxon, 1980).

3.4.6 Rumen Fermentation Characteristics

Increasing dietary NFC reduced rumen pH during the higher concentrate feeding period ($P = 0.01$; Table 3.5). A treatment×time interaction was observed as rumen pH for heifers fed HNFC significantly declined from d 0 to d 28 ($P < 0.01$) and remained lower than heifers fed LNFC ($P = 0.03$) and LNFC+ ($P = 0.04$) on d 56. Rumen pH remained similar from d 0 to d 56 for heifers fed either LNFC diet. Following a diet adjustment, rumen pH increased significantly over time for all treatments in response to increased forage inclusion, as anticipated. Rumen pH, on average, was similar among treatments ($P = 0.48$). A treatment×time effect was observed ($P < 0.01$), as heifers fed HNFC exhibited the greatest response in magnitude, with rumen pH increasing 10.7% compared to 7.6% and 5.2% for LNFC+ and LNFC, respectively, from d 56 to d 84 (Figure 3.8). This likely reflects a potential negative effect of an abrupt transition for heifers fed HNFC to a diet with less readily fermentable carbohydrates as FE was reduced for HNFC compared to LNFC immediately following a diet change. Rumen NH3 concentrations were similar among treatments throughout the study and above 5.0 mg/dL, indicating efficient utilization of N for microbial CP synthesis (Satter and Slyter, 1974) and also agreeing with PUN concentrations described above. As DMI were similar among treatments during the higher concentrate feeding period but were greatest for

heifers fed HNFC during the grower period, we would expect a relative increase in rumen NH3 for heifers fed HNFC during the grower period due to increased CP intake. However, similar to PUN, we did not observe an overall treatment or treatment×time effect for rumen NH3 concentrations. Total VFA concentrations were similar among treatments during the higher concentrate feeding period. Similarly, the overall rumen VFA profile was not significantly altered by dietary NFC during the higher concentrate feeding period, with the exception of valerate proportions being significantly higher for both LNFC diets compared to HNFC ($P = 0.01$). Following an increase in forage inclusion, total VFA concentrations were similar among treatments ($P = 0.13$) and rumen fermentation profiles were significantly altered in favor of lower proportions of acetate ($P < 0.01$), higher proportions of butyrate ($P < 0.01$), higher proportions of isoacids ($P = 0.04$), and lower A:P ratio ($P = 0.02$) for heifers fed HNFC compared to LNFC. Reduced rumen pH and altered VFA profiles with increasing dietary NFC agree with findings reported by Batajoo and Shaver (1994) in lactating dairy cows who observed that as dietary NFC increased from 24% to 42% of the dietary DM (47.5% alfalfa silage diet), molar proportions of butyrate linearly increased and pH, molar proportions of acetate, and A:P ratio linearly decreased. Similarly, Lascano and Heinrichs (2009) found as dietary concentrate increased from 20 to 60% on a DM basis in corn silage-based diets for dairy heifers, molar proportions of acetate decreased linearly. Even though molar proportions of propionate were numerically higher for HNFC compared to LNFC during the lower F:C feeding period, mean proportions were not statistically different throughout the study ($P > 0.10$). Feeding higher starch diets to ruminants usually results in greater ruminal concentrations of propionate, increasing the potential for gluconeogenesis and

thereby increasing circulating glucose concentrations. However, as glucose concentrations were elevated for heifers fed LNFC+ on d 28 and d 84 despite similar proportions of propionate across treatments, shifts in rumen fermentation patterns due to diet composition may not be consistently indicative of whole-body glucose metabolism, as suggested by Harmon (1992). Butyrate concentrations increased at the expense of acetate for heifers fed HNFC during the grower period, agreeing with results from Ipharraguerre et al. (2002) where soybean hulls replaced corn in lactating cow diets resulting in diets ranging from 15.6% to 35.9% NSC on a DM basis. Other studies replacing energy from starch with NDF have also exhibited similar VFA profiles to those observed during the grower period (Sarwar et al., 1991; Sarwar et al., 1992; Grigsby et al., 1993). Increased ruminal butyrate concentrations often observed when greater concentrations of starch are fed have the potential to influence rumen development as butyrate stimulates papillae development in growing ruminants (Baldwin et al., 2004). Additionally, VFA absorption across the rumen epithelium is markedly greater for concentrate-fed compared to forage-fed sheep (Gäbel et al., 1991), which indicates diets with high fermentability have the potential to increase the capacity of the rumen epithelium to absorb VFA (Gäbel et al., 2002; Penner et al., 2011). It is unclear why rumen fermentation was altered in the grower period and not in the lower F:C feeding period, as dietary forage inclusion was increased, thereby reducing NFC for all treatments in the grower period. It is possible that increased DMI in the grower period influenced the fermentation profile as more substrate was available for microbial degradation, though total VFA concentrations were lower in this period compared to the lower F:C feeding period. Additionally, as total VFA concentrations were similar among treatments

and ADG and skeletal growth were not improved for heifers fed HNFC, it appears that, under the conditions of this study, diet fermentability may not influence performance as a function of VFA absorption and utilization.

3.5 Summary and Conclusions

When evaluating performance of prepubertal dairy heifers offered diets with altered carbohydrate profiles and energy content, weight gain and skeletal growth rates were enhanced for heifers consuming higher energy diets (high NFC and low NFC with added fat). Improvements in performance were greater for heifers fed low NFC with added fat when dietary F:C ratios were 35:65 compared to 60:40. As DMI were similar among treatments during the higher concentrate feeding period, feed efficiency improved 8 to 11% for heifers fed low NFC with added fat compared to other treatments. Overall, DM intake increased and NDF intake decreased as a percent of BW for heifers fed high NFC diets compared to low NFC diets, suggesting intake regulation for heifers between 4.5 and 8.5 mo of age may be determined more by physical restriction than chemical signaling. Surprisingly, rumen fermentation was not significantly altered by carbohydrate profile when F:C ratios were lower, suggesting that, under the conditions of this study, energy availability played a larger role in improving growth rates than diet fermentability. Feed costs per kg of gain increased 15 to 20% for a high NFC diet compared to low NFC diet with added fat, suggesting adding fat to diets containing high fiber by-product feeds may be a cost-effective strategy for feeding growing heifers.

3.6 Acknowledgements

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Table 3.1. Ingredient and nutrient analysis (± s.d.) of diets fed during throughout the study.

	35:65[1]			Grower (60:40[1])		
Item[2]	HNFC	LNFC	LNFC+	HNFC	LNFC	LNFC+
Ingredient, % of DM						
Alfalfa/orchardgrass hay	35.0	35.0	35.0	60.0	60.0	60.0
Cracked corn	--	14.6	14.6	--	9.0	9.0
Ground corn	32.5	--	--	20.0	--	--
SBM	8.4	6.1	6.1	5.2	3.8	3.8
DDGS	6.7	6.7	6.7	4.1	4.1	4.1
Soybean hulls	7.3	9.0	11.8	4.5	5.5	7.2
Wheat middlings	--	11.2	11.2	--	6.9	6.9
Cottonseed hulls	9.0	16.3	10.7	5.5	10.0	6.6
Stabilized fat blend[3]	--	--	2.8	--	--	1.7
Mineral premix[4]	1.1	1.1	1.1	0.7	0.7	0.7
Nutrient composition[5]						
DM	90.7 (0.0)	91.5 (0.1)	91.0 (0.1)	91.4 (0.0)	91.5 (0.2)	91.6 (0.0)
ME[6], Mcal/kg	2.71 (0.00)	2.61 (0.00)	2.77 (0.02)	2.51 (0.00)	2.43 (0.05)	2.50 (0.03)
NE_m[7], Mcal/kg	1.79 (0.00)	1.70 (0.00)	1.84 (0.02)	1.61 (0.00)	1.54 (0.04)	1.60 (0.02)
NE_g[8], Mcal/kg	1.17 (0.00)	1.09 (0.00)	1.21 (0.02)	1.01 (0.00)	0.95 (0.04)	1.00 (0.02)
TDN	71.0 (0.0)	68.8 (0.0)	72.3 (0.5)	67.0 (0.0)	64.6 (1.1)	66.2 (0.6)
CP	16.7 (0.0)	17.4 (0.3)	16.7 (0.4)	16.3 (0.0)	16.5 (0.0)	16.4 (0.2)
NFC[9]	37.5 (0.0)	29.4 (0.5)	28.4 (1.3)	35.7 (0.0)	29.3 (1.9)	27.3 (1.0)
Starch	23.1 (0.7)	13.2 (0.6)	12.9 (0.0)	14.5 (0.8)	9.1 (0.0)	7.9 (1.4)
NDF	35.3 (0.0)	43.7 (0.1)	43.3 (1.8)	40.3 (0.0)	46.8 (2.3)	47.4 (0.8)
Forage NDF	18.4	18.4	18.4	31.0	31.0	31.0
ADF	24.2 (0.0)	28.4 (0.1)	28.3 (2.3)	28.0 (0.0)	32.0 (1.6)	32.4 (0.4)
Crude fat	3.7 (0.0)	3.8 (0.2)	6.7 (0.1)	3.2 (0.0)	2.9 (0.4)	4.3 (0.1)
Ca	0.91 (0.00)	0.96 (0.02)	0.90 (0.01)	1.03 (0.00)	1.08 (0.01)	1.09 (0.01)
P	0.45 (0.00)	0.55 (0.00)	0.48 (0.04)	0.36 (0.00)	0.42 (0.00)	0.41 (0.01)

[1]Forage:concentrate ratio on a DM basis.
[2]HNFC = high non-fiber carbohydrate (NFC); LNFC = low NFC; LNFC+ = low NFC with added fat.
[3]Sourced from Griffin Industries (Russellville, KY) as a fat product (feed grade) containing > 90% total fatty acids, < 20% free fatty acids, and < 1.0% moisture on an as-fed basis.
[4]Sourced from Kent Feeds (Muscatine, IA) containing 14.0% Ca, 6.5% P, 2.0% Mg, 2.0% K, 8.3% Na, 12.7% Cl, 900 ppm Cu, 1700 ppm Mn, 20 ppm Se, and 4700 ppm Zn on an as-fed basis.
[5]All values given as a percent of DM unless otherwise stated.
[6]Estimated using following equation: $ME = 1.01 \times [(0.04409 \times TDN) - 0.45]$.
[7]Estimated using following equation: $NE_m = (1.37 \times ME) - (0.138 \times ME^2) + (0.0105 \times ME^3) - 1.12$.
[8]Estimated using following equation: $NE_g = (1.42 \times ME) - (0.174 \times ME^2) + (0.0122 \times ME^3) - 1.65$.
[9]NFC estimated using following equation: NFC = 100 – NDF – CP – Crude fat – Ash.

Table 3.2. Weight and skeletal growth responses of prepubertal dairy heifers fed diets containing high non-fiber carbohydrate (NFC), low NFC (LNFC), or LNFC with added fat (LNFC+) grain mixes.

					P-value[1]	
Item	HNFC	LNFC	LNFC+	SEM	T	T×S
Body weight, kg						
d 0	145.5	145.5	145.4	1.88	--	--
d 56	198.4^{ab,y}	195.6^{b}	203.0^{a,x}	1.88	0.02	--
d 112	250.5^{ab,y}	246.5^{b}	255.3^{a,x}	1.88	< 0.01	--
Total BW gain, kg	105.1^{ab}	101.2^{b}	109.9^{a}	2.84	0.13	--
ADG[2], kg/d						
d 0 to 56	0.97^{b}	0.92^{b}	1.06^{a}	0.028	0.02	0.01
d 56 to 112	0.93	0.91	0.93	0.033	0.86	< 0.01
d 0 to 112	0.95^{ab}	0.91^{b}	1.00^{a}	0.026	0.13	< 0.01
Hip height, cm						
d 0	106.8	106.9	106.9	0.34	--	--
d 56	113.9^{ab}	113.5^{b}	114.6^{a}	0.34	0.06	--
d 112	120.9^{a}	119.9^{b}	121.8^{a}	0.34	< 0.01	--
Monthly gain	3.5^{ab}	3.3^{b}	3.7^{a}	0.14	0.10	0.40
Withers height, cm						
d 0	101.8	101.8	101.7	0.38	--	--
d 56	108.4^{ab}	107.9^{b}	109.2^{a}	0.38	0.06	--
d 112	115.3^{b}	114.9^{b}	116.9^{a}	0.38	< 0.01	--
Monthly gain	3.4^{ab,y}	3.3^{b}	3.8^{a,x}	0.15	0.07	0.45
Hip width, cm						
d 0	28.5	28.5	28.5	0.21	--	--
d 56	32.2^{b}	32.8^{a}	32.7^{a}	0.21	0.07	--
d 112	35.5	35.4	35.7	0.21	0.70	--
Monthly gain	1.8	1.7	1.8	0.08	0.82	0.02
Heart girth, cm						
d 0	120.7	120.7	120.8	0.52	--	--
d 56	132.5	131.6	132.9	0.53	0.16	--
d 112	146.6	145.6	146.1	0.52	0.40	--
Monthly gain	6.5	6.4	6.5	0.26	0.90	0.70
BCS[3], 1 to 5 scale						
d 0	2.64	2.66	2.65	0.021	--	--
d 56	2.79^{b}	2.76^{b}	2.85^{a}	0.021	< 0.01	--
d 112	2.83^{a}	2.75^{b,y}	2.81^{a,x}	0.021	0.03	--

[1]T = treatment effect; T×S = treatment×time interaction.
[2]Average daily gain.
[3]Body condition score.
[ab]Means differ at $P \leq 0.05$ level.
[xy]Means tend to differ at $0.10 \geq P > 0.05$.

Table 3.3. Intake and feed efficiency of prepubertal dairy heifers fed diets containing high non-fiber carbohydrate (NFC), low NFC (LNFC), or LNFC with added fat (LNFC+) grain mixes.

					P-value[1]	
Item	HNFC	LNFC	LNFC+	SEM	T	T×S
DM intake, kg/d						
d 0 to 56	5.75	5.72	5.83	0.066	0.45	0.01
d 56 to 112	7.50[a]	6.94[b]	7.00[b]	0.160	0.06	< 0.01
d 0 to 112	6.62	6.33	6.42	0.102	0.15	< 0.01
DM intake, % of BW						
d 0 to 56	3.26	3.24	3.22	0.038	0.73	0.03
d 56 to 112	3.25[a]	3.03[b]	2.96[b]	0.045	< 0.01	< 0.01
d 0 to 112	3.25[a]	3.14[b]	3.09[b]	0.032	< 0.01	< 0.01
NDF intake, kg/d						
d 0 to 56	2.03[b]	2.51[a]	2.58[a]	0.028	< 0.01	< 0.01
d 56 to 112	3.10	3.24	3.28	0.071	0.23	< 0.01
d 0 to 112	2.57[b]	2.87[a]	2.93[a]	0.046	< 0.01	< 0.01
NDF intake, % of BW						
d 0 to 56	1.15[b]	1.42[a]	1.42[a]	0.015	< 0.01	< 0.01
d 56 to 112	1.34[b]	1.41[a]	1.39[a]	0.021	0.09	< 0.01
d 0 to 112	1.25[b]	1.42[a]	1.41[a]	0.014	< 0.01	< 0.01
ME intake, Mcal/d						
d 0 to 56	16.2[ab,y]	15.6[b]	16.7[a,x]	0.18	< 0.01	< 0.01
d 56 to 112	19.6[a,x]	17.7[b]	18.4[ab,y]	0.42	0.03	< 0.01
d 0 to 112	17.9[a]	16.7[b]	17.6[a]	0.27	0.02	< 0.01
Feed efficiency[2]						
d 0 to 56	0.166[ab,y]	0.161[b]	0.181[a,x]	0.006	0.06	0.20
d 56 to 112	0.123	0.132	0.133	0.007	0.52	0.10
d 0 to 112	0.144	0.146	0.157	0.004	0.12	0.07
ME efficiency[3]						
d 0 to 112	0.053	0.055	0.057	0.002	0.26	0.10
CP efficiency[4]						
d 0 to 112	0.874[ab,y]	0.862[b]	0.948[a,x]	0.027	0.08	0.05

[1]T = treatment effect; T×S = treatment×time interaction.
[2]Feed efficiency expressed as kg of ADG per kg of daily DM intake.
[3]Nutrient efficiency expressed as kg of ADG per Mcal of daily ME intake.
[4]Nutrient efficiency expressed as kg of ADG per kg of daily CP intake.
[ab]Means differ at $P \leq 0.05$ level.
[xy]Means tend to differ at $0.10 \geq P > 0.05$ level.

Table 3.4. Daily feed costs for heifers fed diets containing high non-fiber carbohydrate (NFC), low NFC (LNFC), or LNFC with added fat (LNFC+) grain mixes.

					P-value[1]	
Item[2]	HNFC	LNFC	LNFC+	SEM	T	T×S
Daily feed cost per hd						
Study costs						
d 0 to 56	1.63[a]	1.49[c]	1.58[b]	0.017	< 0.01	< 0.01
d 57 to 112	1.83[a]	1.59[b]	1.65[b]	0.036	< 0.01	< 0.01
d 0 to 112	1.73[a]	1.54[c]	1.61[b]	0.023	< 0.01	< 0.01
5 yr average costs[3]						
d 0 to 56	1.89[a]	1.58[c]	1.72[b]	0.019	< 0.01	< 0.01
d 57 to 112	2.07[a]	1.69[b]	1.79[b]	0.040	< 0.01	< 0.01
d 0 to 112	1.98[a]	1.64[b]	1.75[b]	0.025	< 0.01	< 0.01
Cost of gain[4]						
Study costs						
d 0 to 56	1.86[a]	1.78[ab]	1.59[b]	0.080	0.09	0.43
d 57 to 112	2.38	2.10	2.16	0.237	0.70	0.93
d 0 to 112	2.12	1.94	1.88	0.125	0.39	0.98
5 yr average costs						
d 0 to 56	2.15[a]	1.88[b]	1.73[b]	0.088	0.02	0.37
d 57 to 112	2.69	2.24	2.35	0.259	0.46	0.92
d 0 to 112	2.42	2.06	2.04	0.137	0.13	0.98

[1]T = treatment effect; T×S = treatment×time interaction.
[2]All values given in US dollars ($).
[3]Calculated from average commodity prices from 2008 to 2013 crop years.
[4]$/kg of average daily gain.
[abc]Means with differing superscripts are significantly different at $P \leq 0.05$ level.

Table 3.5. Blood metabolites and rumen fermentation parameters of prepubertal dairy heifers fed diets containing high non-fiber carbohydrate (NFC), low NFC (LNFC), or LNFC with added fat (LNFC+) grain mixes.

					P-value[1]	
Item	HNFC	LNFC	LNFC+	SEM	T	T×S
Plasma glucose, mg/dL						
d 0 to 56	75.4	75.1	77.9	0.93	0.12	0.09
d 57 to 112	73.2	73.5	73.4	0.79	0.97	0.05
Plasma urea N, mg/dL						
d 0 to 56	10.7	10.7	10.6	0.24	0.88	0.78
d 57 to 112	11.2	10.9	11.1	0.24	0.78	0.71
Rumen pH						
d 0 to 56	5.96[b]	6.18[a]	6.18[a]	0.055	0.01	0.03
d 57 to 112	6.44	6.36	6.37	0.049	0.48	0.01
Rumen NH3, mg/dL						
d 0 to 56	7.29	7.77	7.81	0.669	0.83	0.39
d 57 to 112	5.70	5.72	6.42	0.328	0.21	0.17
Total VFA[2], mM						
d 0 to 56	94.9	84.7	91.2	4.36	0.26	0.66
d 57 to 112	74.9	69.3	63.3	3.89	0.13	0.20
Molar proportion of VFA[3]						
d 0 to 56						
Acetate	65.4	66.5	66.8	0.62	0.29	0.13
Propionate	24.3	22.7	23.2	0.59	0.17	0.32
Butyrate	7.3	7.0	7.3	0.26	0.65	0.21
Valerate	0.7[b]	0.8[a]	0.8[a]	0.02	< 0.01	0.05
Isoacids[4]	2.3	2.6	2.4	0.11	0.28	0.95
A:P[5]	2.75	3.01	2.93	0.108	0.21	0.23
d 57 to 112						
Acetate	69.1[b]	71.8[a,x]	70.5[a,y]	0.47	< 0.01	0.08
Propionate	20.0	18.9	19.9	0.44	0.16	0.62
Butyrate	8.0[a]	6.8[b]	7.1[b]	0.17	< 0.01	0.03
Valerate	0.7	0.7	0.8	0.03	0.30	0.01
Isoacids	2.1[a,x]	1.8[ab,y]	1.7[b]	0.10	0.04	< 0.01
A:P	3.56[b]	3.93[a]	3.64[ab]	0.104	0.02	0.17

[1]T = treatment effect; T×S = treatment×time interaction.
[2]Volatile fatty acids.
[3]Molar proportion expressed as mol individual VFA/100 mol total VFA.
[4]Sum of isovalerate and isobutyrate molar proportions.
[5]Acetate:propionate ratio.
[ab]Means differ at $P \leq 0.05$ level.
[xy]Means tend to differ at $0.10 \geq P > 0.05$ level.

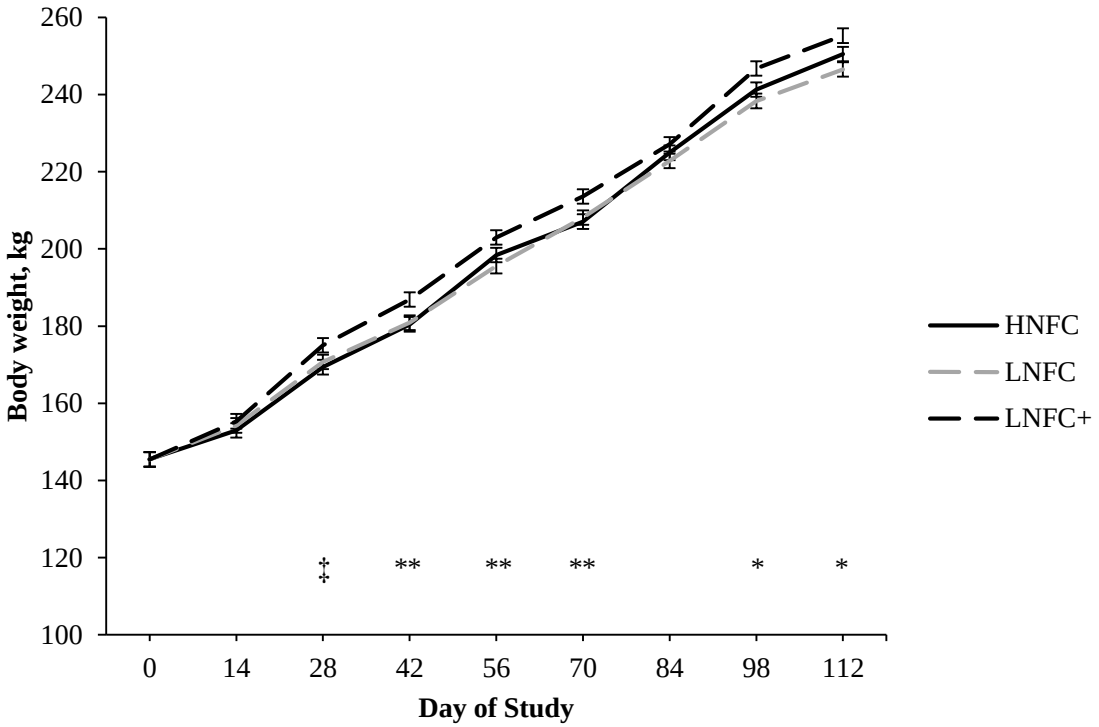

Figure 3.1. Effects of feeding high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) diets on body weight over time. Heifers fed LNFC+ were heaviest on average compared to heifers fed HNFC and LNFC ($P = 0.03$). ‡$0.10 \leq P < 0.05$; **$P \leq 0.05$; *$P \leq 0.01$.

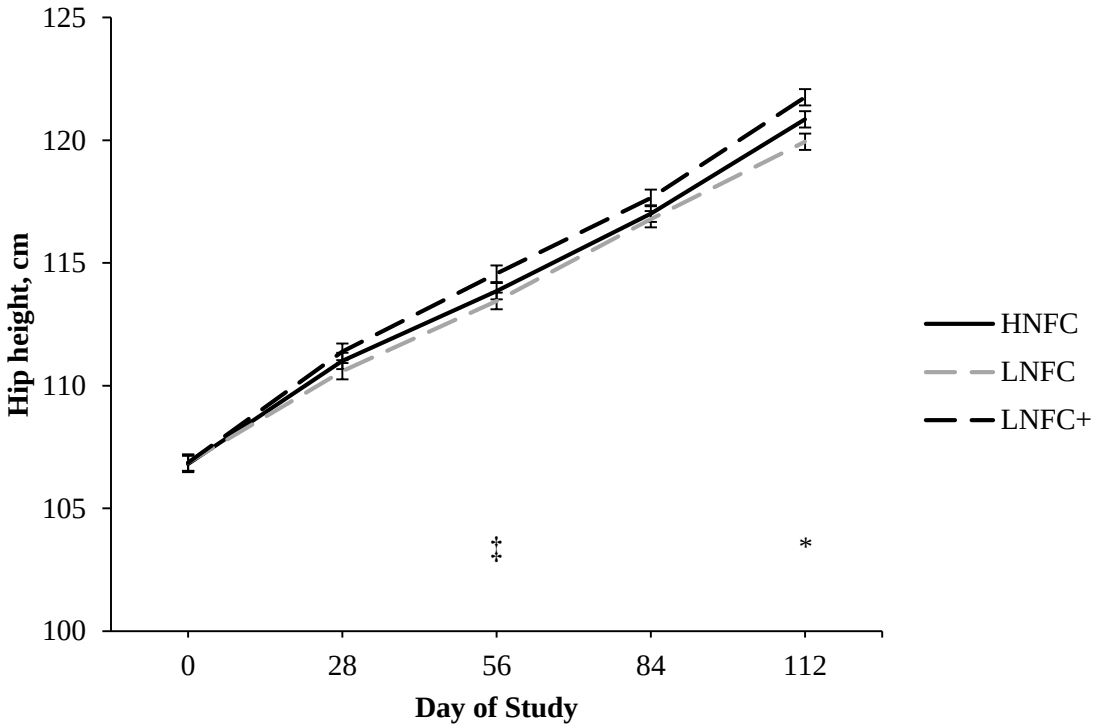

Figure 3.2. Effects of feeding high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) diets on hip height over time. Heifers fed LNFC+ were taller on average compared to heifers fed LNFC ($P = 0.02$). ‡$0.10 \leq P < 0.05$; *$P \leq 0.01$.

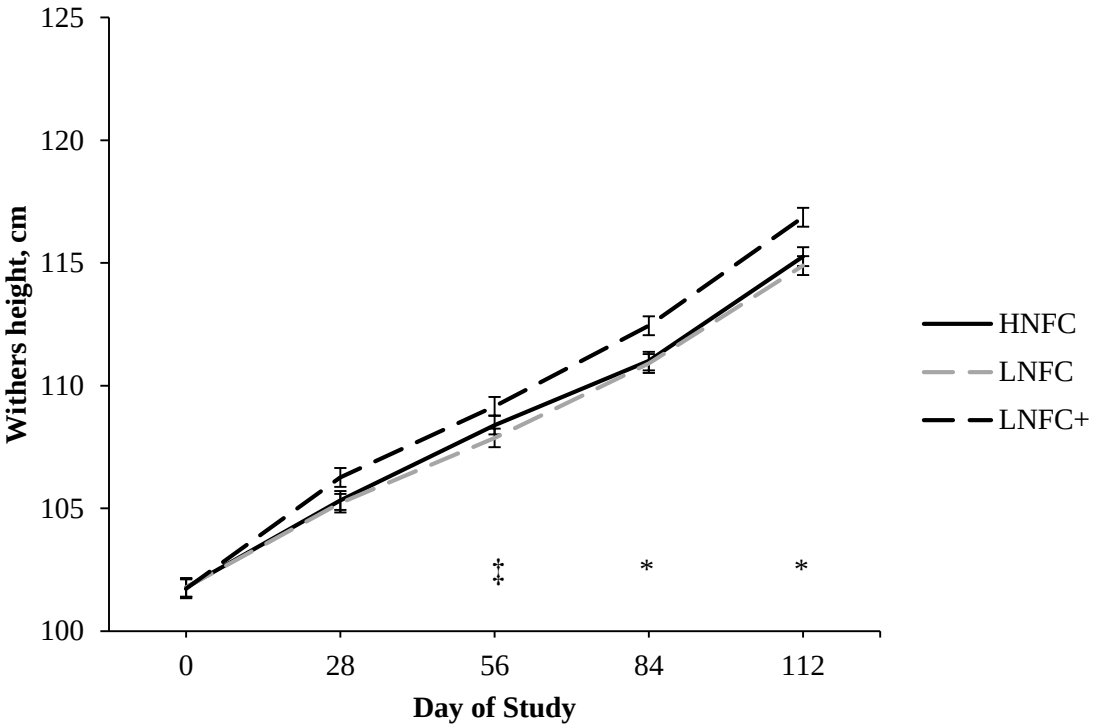

Figure 3.3. Effects of feeding high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) diets on withers height over time. Heifers fed LNFC+ were taller on average compared to heifers fed LNFC and HNFC ($P = 0.03$). ‡$0.10 \leq P < 0.05$; *$P \leq 0.01$.

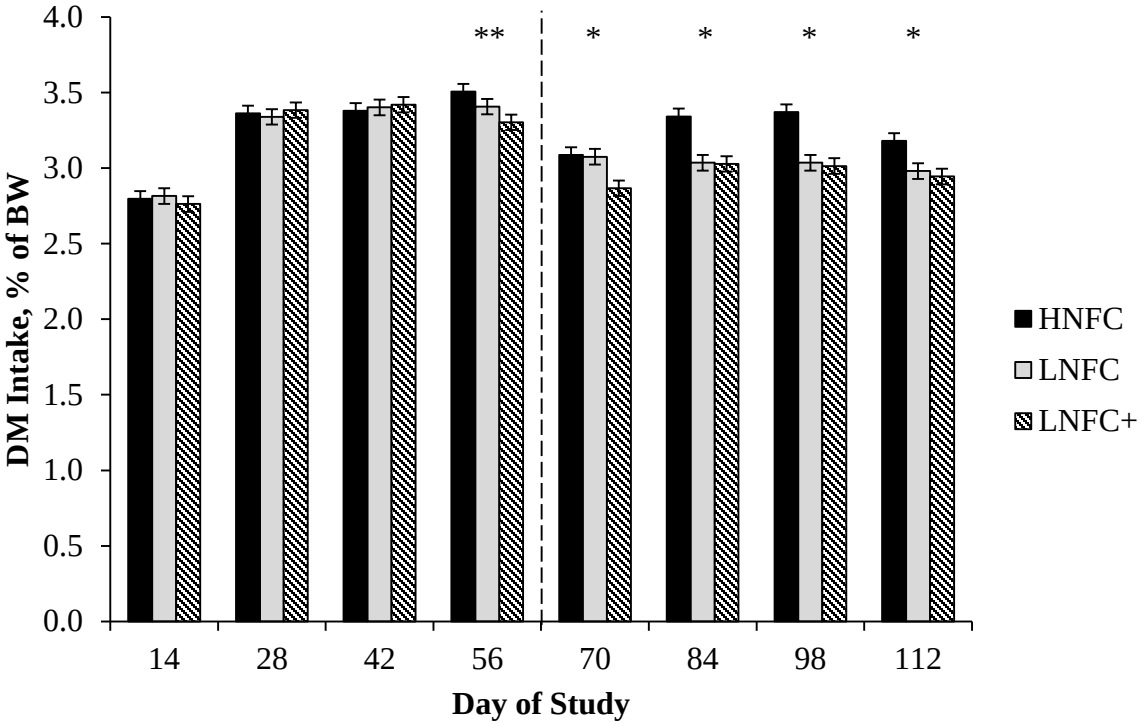

Figure 3.4. Effects of feeding high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) diets on DM intake as a percent of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Heifers fed HNFC had greater overall DM intake compared to heifers fed LNFC or LNFC+ ($P < 0.01$). A treatment×time interaction was observed, as DM intake was similar among treatments until d 56, and then heifers fed HNFC maintained the greatest DM intake throughout the remainder of the study ($P < 0.01$). **$P \leq 0.05$; *$P \leq 0.01$.

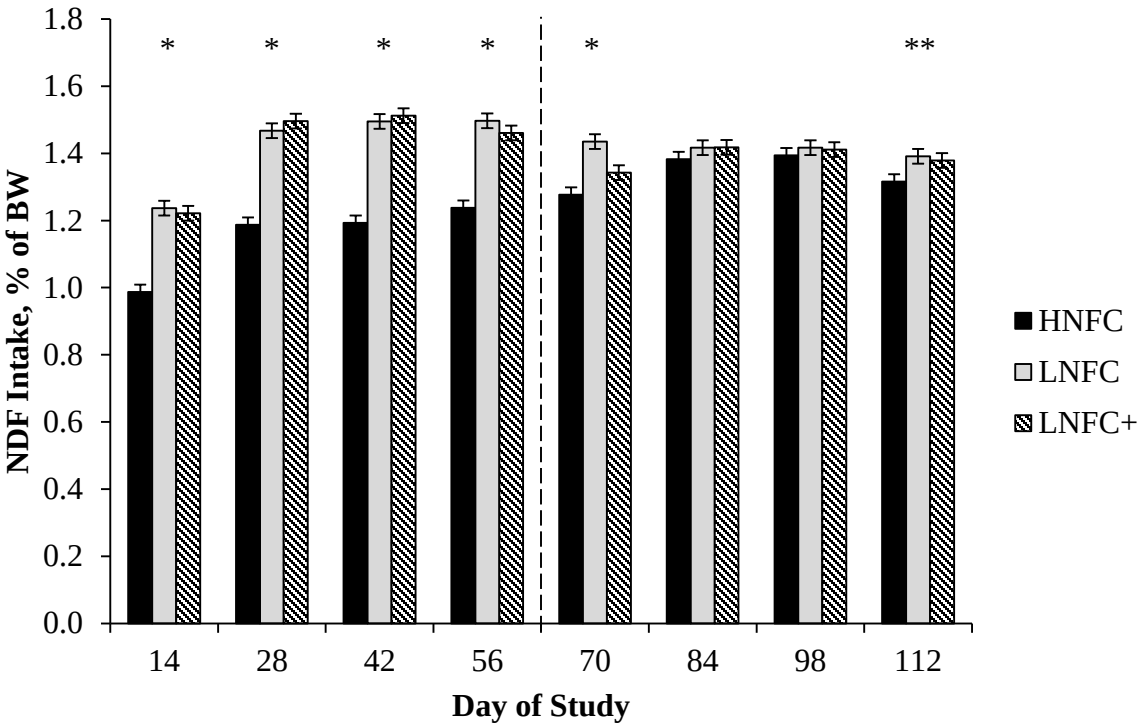

Figure 3.5. Effects of feeding high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) diets on total NDF intake as a percent of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Total NDF intake increased as NFC decreased in the diet ($P < 0.01$). A treatment×time interaction was observed ($P < 0.01$), as total NDF intake as a percent of BW was similar among treatments on d 84 ($P = 0.43$) and d 98 ($P = 0.75$), but increased for heifers fed LNFC or LNFC+ compared to HNFC on d 112 ($P = 0.04$). $^{**}P \leq 0.05$; $^{*}P \leq 0.01$.

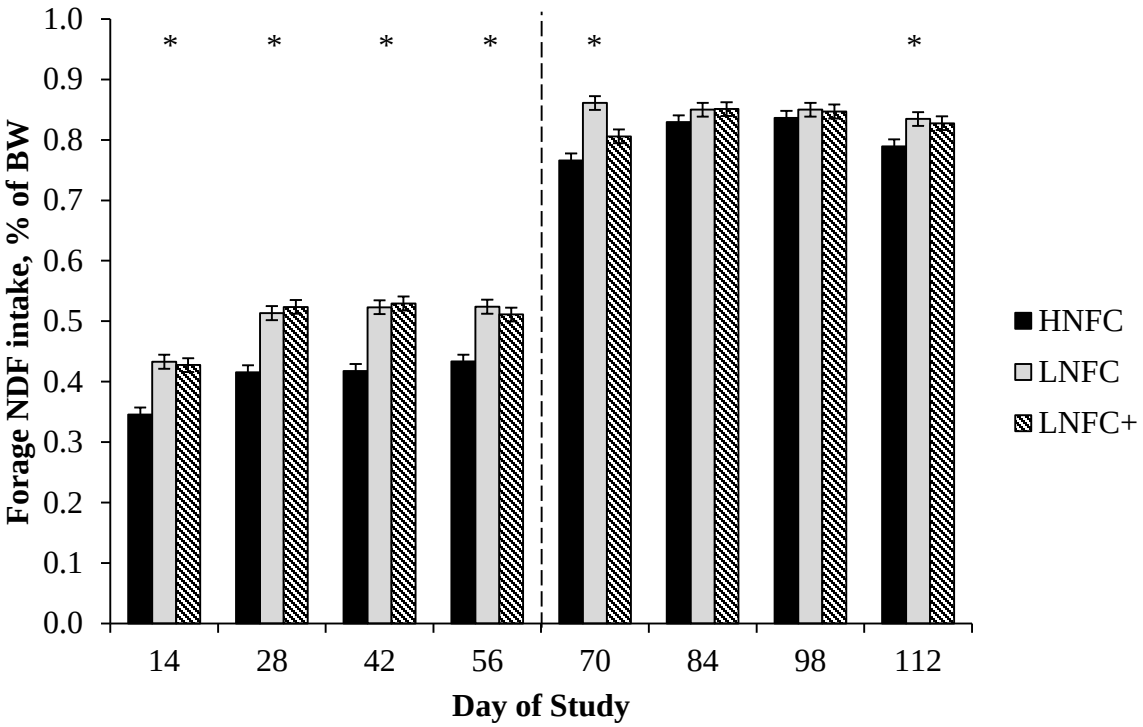

Figure 3.6. Effects of feeding high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) diets on forage NDF intake as a percent of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Forage NDF was a greater proportion of total NDF intake and forage NDF intake increased as NFC decreased in the diet during the first 56 d ($P < 0.01$); however, a treatment×time interaction was observed overall ($P < 0.01$), as forage NDF intake was similar among treatments on d 84 ($P = 0.33$) and d 98 ($P = 0.69$), but increased for heifers fed LNFC or LNFC+ compared to HNFC on d 70 and before and on d 112 ($P = 0.01$). *$P \leq 0.01$.

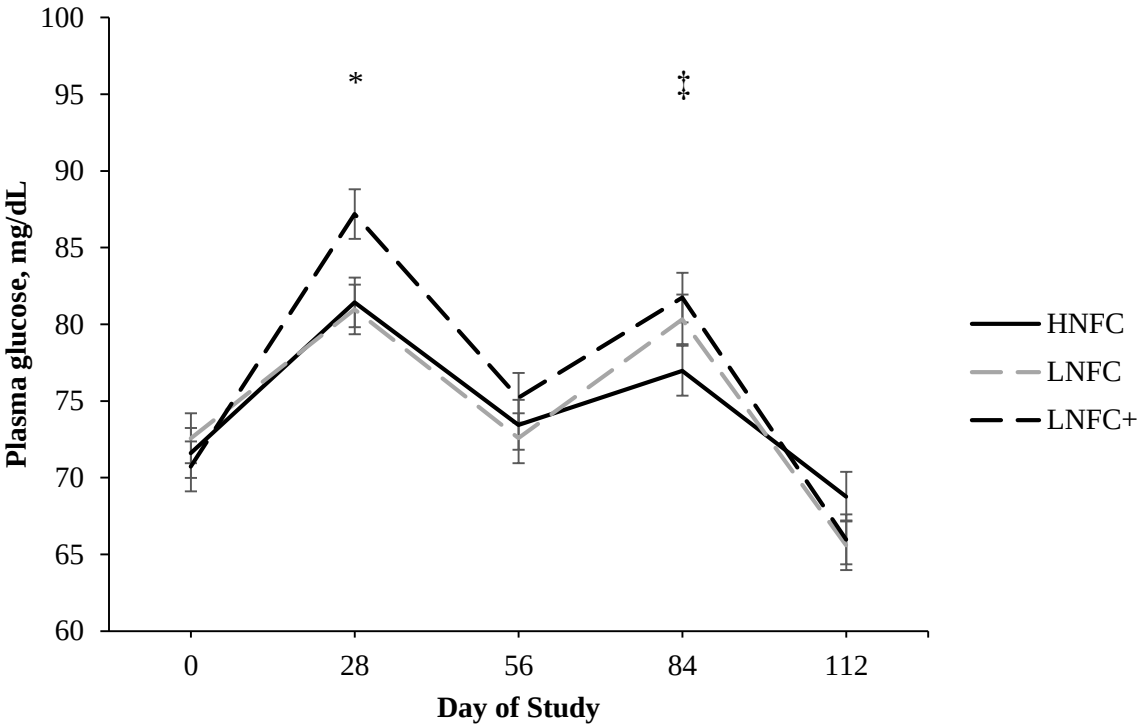

Figure 3.7. Plasma glucose responses to feeding high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) diets to prepubertal dairy heifers over time. No overall effect of treatment was detected ($P = 0.31$); however, a tendency for a treatment×time interaction was observed ($P = 0.09$) as heifers fed LNFC+ had elevated glucose concentrations on d 28 ($P = 0.01$) and d 84 ($P = 0.04$) compared to heifers fed HNFC. ‡$0.10 \leq P < 0.05$; *$P \leq 0.01$.

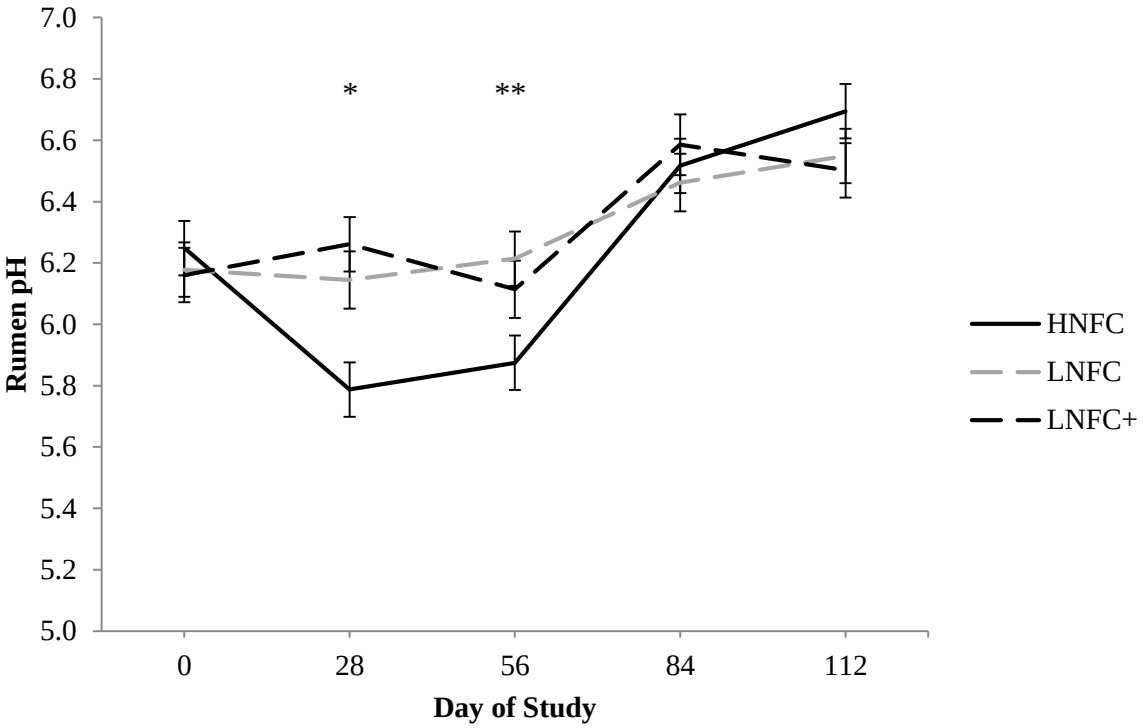

Figure 3.8. Rumen pH responses to feeding high non-fiber carbohydrate (HNFC), low NFC, LNFC with added fat (LNFC+) diets to prepubertal dairy heifers over time. No overall effect of treatment was detected ($P = 0.21$); however, a treatment×time interaction was observed ($P < 0.01$) as heifers fed HNFC had lower rumen pH on d 28 ($P < 0.01$) and d 56 ($P = 0.02$) compared to heifers fed LNFC and LNFC+. $^{**}P \leq 0.05$; $^{*}P \leq 0.01$.

CHAPTER 4. IMPACT OF DIETARY CONCENTRATE INCLUSION ON GROWTH PERFORMANCE, BLOOD METABOLITES, AND RUMEN FERMENTATION CHARACTERISTICS OF PREPUBERTAL DAIRY HEIFERS

4.1 Abstract

The objective of this study was to evaluate the impact of decreasing dietary forage:concentrate (F:C) ratio on growth, dry matter intake (DMI), feed efficiency (G:F), and rumen fermentation characteristics of prepubertal dairy heifers. Seventy-eight Holstein heifers (133.1 ± 24.3 kg, 125 ± 22 d of age) were randomly allocated by body weight (BW) to 1 of 15 pens. Pens were randomly assigned to dietary treatments balanced for CP and ME containing F:C ratios of 20:80, 40:60, or 60:40 and fed for 56 d. Following the treatment period, all pens were switched to a common diet (60:40 F:C ratio) and fed for an additional 56 d. Body weights were collected every 2 wk, and skeletal growth and body condition score (BCS) were measured monthly. Rumen fluid was collected esophageally 6 hr after feeding from 10 heifers per treatment (2 heifers/pen) to determine rumen pH, NH3, and volatile fatty acids (VFA) monthly. Heifers fed 20:80 were 13.7 and 27.1 kg heavier than 40:60 and 60:40, respectively, at the end of the treatment period. Similarly, ADG, DMI, G:F, and skeletal growth increased linearly with increasing concentrate inclusion during the treatment period. There was a treatment×time interaction for DMI (percent of BW), with 20:80 consuming 3.4 and 3.0% of BW compared to 60:40 consuming 2.8 and 3.3% of BW

on d 56 and 112, respectively. Total NDF intake was 26.7% greater for 20:80 compared to 60:40 during the treatment period, but similar among treatments during the grower period. During the grower period, ADG was increased for heifers fed 40:60 and 60:40 compared to 20:80 on d 70 and 84. Heifers fed 40:60 exhibited improved G:F on d 84 of the grower period compared to 20:80, with 40:60 averaging 0.166 compared to 0.125 kg ADG/kg DMI for 20:80. Skeletal growth rates were similar between treatments during the common period from d 56 to 112, resulting in an overall increase in frame size for heifers fed 20:80 in the treatment period. Feeding greater concentrate inclusion rates increased BCS during the treatment period from 2.49 for 60:40 to 2.78 for 20:80. Heifers fed 20:80 had greater plasma urea N (PUN) and glucose than 40:60 and 60:40 during the treatment period; however, PUN and glucose were similar among treatments during the grower period. During the treatment period, total VFA concentrations were greater for 20:80 and 40:60 than 60:40 on d 56, averaging 118.2, 108.1, and 74.2 mM, respectively. Molar proportions of acetate and A:P ratio were greatest and molar proportions of propionate and butyrate were least for 60:40 on d 56 of the treatment period compared to 20:80. After being placed on a common diet, total VFA and molar proportions of VFA were similar between treatments. Rumen pH was least for 20:80 and greatest for 60:40 on d 56, but similar during the common period. Growing dairy heifers had greater ADG when fed high amounts of concentrate, but ADG and G:F were reduced compared with heifers fed moderate to low amounts of concentrate after switching to a high-forage diet.

4.2 Introduction

Heifer development in the dairy industry is an expensive enterprise considering costs associated with feeding, management, and no return on investment until the replacement heifer enters lactation. Feed management factors imposed during the growing period can influence future potential for milk production and longevity, including feeding for increased pre-weaning growth rates (Soberon and Van Amburgh, 2013), encouraging increased DMI at weaning (Heinrichs and Heinrichs, 2011), and providing lower energy diets to prevent over-conditioning and excess fat deposition in the mammary gland (Radcliff et al., 1997; Radcliff et al., 2000; Brown et al., 2005a). Optimizing growth rates and intake usually increases feed costs, which over the last 20 yr have increased from 60.3% (Gabler et al., 2000) to 73.0% (Heinrichs et al., 2013) of the average total cost to raise a heifer from birth to first calving. Strategies to reduce feed costs without sacrificing health and productivity of growing heifers warrant exploring, as data is limited for heifers from weaning to puberty.

Improving feed efficiency of growing heifers post-weaning can potentially reduce raising costs. Weaned dairy heifers typically receive forage-based diets, which often results in lower feed efficiency due to intake of poorly digestible fiber. Replacing forages with highly digestible concentrate sources has been shown to increase dietary DM digestibility and N retention (Moody et al., 2007), as well as feed efficiency (Zanton and Heinrichs, 2007) for growing dairy heifers. Additionally, when growing beef heifers were fed 75% concentrate versus 75% alfalfa hay at equal ME intake, tissue energy retention was greater and heat energy expenditure was reduced (Reynolds et al., 1991). However, when fed for *ad libitum* intake from 3 mo of age to breeding, high-concentrate

diets containing 1.2 Mcal/kg of NE_g have been shown to reduce first lactation milk production compared to a 90% forage diet containing 0.8 Mcal/kg of NE_g (Radcliff et al., 2000). Forage is required for maintaining rumen health and is an inexpensive source of energy per unit of DM, making forage a popular ingredient in heifer diets. Diets with reduced inclusion of forages in favor of higher concentrate levels are often more expensive to feed per kg of DM (Dickerson, 1978), but costs are highly dependent on variability in forage and commodity prices relative to dietary inclusion (Berthiaume et al., 2006). However, costs per kg of DM do not factor in differences in animal performance that may result in less overall expense to feed a heifer to a common weight.

Metabolic changes have been recognized in beef cattle abruptly switched to high concentrate diets from high forage diets, mostly due to reduced feed intake (Brown et al., 2000), changes in microbial ecology (Tajima et al., 2001; Fernando et al., 2010), and disrupted rumen function (Loerch and Fluharty, 1999). Abrupt diet changes resulting in greater forage inclusion would likely affect intake and rumen fermentation, particularly in developing heifers. As concentrates are increased in the diet, subsequent reductions in pH are expected as microbial ecology shifts to accommodate new fermentation substrates. When steers (Fulton et al., 1979; Lyle et al., 1981) or sheep (Mackie and Gilchrist, 1979) were gradually adapted to high-concentrate from high-forage diets, rumen pH declined over time and concentrations of propionate and lactate increased. Abruptly increasing from a 25% to 96% concentrate diet for dry Holstein cows resulted in reduced rumen pH and acetate concentrations 14 d after the diet change; however, considerable animal-to-animal variation precluded any statistical differences in other VFA concentrations between low and high grain diets over the 4 wk trial (Tajima et al.,

2001). In contrast, when dry Holstein cows were fed a high energy (32% NDF, 44% NFC, and 1.70 Mcal/kg NE_l) close-up diet, rumen fermentation characteristics were similar to those of cows fed a low energy (40% NDF, 38% NFC, and 1.57 Mcal/kg NE_l) close-up diet after switching to a high energy (25% NDF, 47% NFC, and 1.63 Mcal/kg NE_l) lactation diet (Rabelo et al., 2003). Adaptation to high concentrate diets using step-up feeding regimens for feedlot cattle has been shown to reduce populations of *B. fibrosolvens* and *F. succinogenes* in favor of increasing populations of *M. elsdenii, S. bovis, S. ruminantium*, and *P. bryantii* (Fernando et al., 2010). It stands to reason the opposite would occur when switching growing heifers to a high-forage diet from a high-concentrate diet, as microbial populations would shift from predominately starch- to fiber-digesting bacteria. Transitioning weaned dairy heifers to higher forage diets is required, yet little data exists on the responses to abrupt changes in diet from high concentrate to high forage. Therefore, the objectives of this study were to evaluate the effects of various forage:concentrate ratios (F:C) in prepubertal dairy heifer diets on growth, feed efficiency, rumen fermentation characteristics, blood metabolites, and feed costs and the subsequent response to a rapid increase in dietary forage inclusion. We hypothesized that lesser dietary concentrate inclusion during the treatment period would reduce growth and feed efficiency compared to higher concentrate inclusion, but subsequent performance would be improved for heifers previously fed lower concentrate diets when all heifers were rapidly switched to a high-forage diet.

4.3 Materials and Methods

4.3.1 Animals and Housing

This study was conducted at the Southern Indiana Purdue Agricultural Center (SIPAC) in Dubois, IN from May 28th to September 18th 2012 using Holstein heifers sourced from Buckeye Heifer Resources of Camden, OH. All animal-related procedures were conducted in compliance with approved protocols from the Purdue Animal Care and Use Committee (PACUC no. 11-048). All heifers were acclimated to facilities and a common diet consisting of a grain mix and alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) hay offered in a 30:70 F:C ratio 12 d prior to initiating the study. Seventy-eight Holstein heifers (133.1 ± 24.3 kg, 125 ± 22 d of age) were weighed on 2 consecutive days at the beginning of the study and randomly assigned by weight to 1 of 15 pens with 5 to 6 heifers per pen. Housing consisted of a naturally ventilated barn with 3.7 m x 21.9 m pens, 3.7 m of feed bunk space, and unrestricted access to water. Pens were covered mid-way by slanted steel roofing and bedded with sawdust throughout the study as needed. Heifers were given magnet boluses and vaccinated 2 wk after beginning the experiment for bovine viral diarrhea, infectious bovine rhinotracheitis, and leptospirosis (Bovi-Shield Gold FP5 L5 HB, Pfizer Animal Health, Kalamazoo, MI) and 7 strains of *Clostridium* (Ultrabac 7, Pfizer Animal Health) and were boostered 4 wk following the first vaccination.

4.3.2 Experimental Design and Treatments

The study was designed with a 56 d treatment period followed by immediate transition to a common diet for all heifers for an additional 56 d grower period. Pens

were randomly assigned to treatment diets containing either 80%, 60%, or 40% concentrate with the remainder of the diet offered as chopped dry hay on a DM basis (20:80, 40:60, and 60:40, respectively). Following the treatment period, all heifers were immediately switched to a common diet with a 60:40 F:C ratio. Feed was delivered as a total mixed ration with concentrate and chopped hay offered once per d at 0700 h during the treatment and grower periods. Ingredient and nutrient composition of grain mixes and forages used in this study are presented in Table 4.1. Diets during each period were formulated according to NRC (2001) recommendations to allow 0.90 kg/d of ADG for growing Holstein heifers. Feed was initially offered at approximately 2.8% of the average pen BW and was adjusted daily to allow for *ad libitum* intake and minimize refusals (<10% daily). Hay used for the treatment diets was harvested at SIPAC in 2011 from a second cutting of an alfalfa/orchardgrass mixture and for the common diet was an alfalfa/orchardgrass purchased off-site. Orts were weighed and sub-sampled once per wk to determine weekly pen intakes. Feed ingredients and orts were dried at 60°C in a forced air oven, ground through a 1.0 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ), composited by month, and analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Labs, Ithaca, NY). Samples were analyzed for CP (AOAC 984.13; AOAC, 1990), NDF (Van Soest et al., 1991), ADF (AOAC 973.18; AOAC, 1990), ME (calculated from TDN in feed; NRC, 2001), and minerals (microwave digestion followed by inductively coupled plasma spectrometry; Isaac and Johnson, 1985).

4.3.3 Data Collection and Analysis

Heifers were weighed every 2 wk during the treatment and grower periods and skeletal growth measurements, including withers height (WH), hip height (HH), heart girth circumference (HGC), and hip width (HW) were assessed monthly. Body condition score (BCS) was assessed monthly on a scale of 1 to 5 (1 = emaciated, 5 = obese; Edmonson et al., 1989) by 2 evaluators and averaged. Blood samples (10 mL) were collected via jugular venipuncture monthly into evacuated blood tubes containing lithium heparin (BD Diagnostics, Franklin Lakes, NJ). Plasma was aspirated following centrifugation (2500 x *g* for 15 min at 4°C) and frozen at -20°C for later analysis. Plasma was analyzed for plasma urea N (PUN; procedure no. 0580; Stanbio Laboratory Inc., San Antonio, TX) and glucose (procedure no. 1070; Stanbio Laboratory Inc.). Rumen fluid was obtained as described by Dennis et al. (2012) on d 0, 28, 56, 84, and 112 using an esophageal tube from 2 heifers in each pen and analyzed for pH, VFA, rumen NH3, *in vitro* cellulose disappearance, and *in vitro* gas production. Rumen fluid pH was immediately determined (model EL2; Mettler-Toledo, Columbus, OH), and two 20 mL samples of fluid were acidified using 25% w/v meta-phosphoric acid (4:1 sample-to-acid ratio) and frozen at -20°C for later analysis. Rumen fluid samples were analyzed for VFA using gas chromatography on a bonded capillary column (Supelco, Bellefonte, PA; Erwin et al., 1961) and for NH3 using the Kjeldahl procedure (FOSS Kjeltec 2300, Hoganas, Sweden; AOAC 984.13, AOAC, 1990). Anaerobic serum tubes (Chemglass Life Sciences, Vineland, NJ) containing 9.0 mL of basal cellulose media (as described in Dennis et al., 2012) were inoculated with 1.0 mL of rumen fluid from each heifer, serially diluted to 10^{-8} dilution in duplicate, and all tubes were incubated at 37°C for 72 hr.

Following incubation, total gas volume was measured and tubes were autoclaved at 125°C for 20 min to cease bacterial digestion. After autoclaving, residual cellulose was processed using the micro-NDF procedure described by Pell and Schofield (1993).

4.3.4 Statistical Analysis

Data were analyzed overall and by period to determine treatment and carryover effects into the grower period. Growth and intake data were analyzed as repeated measures (Littell et al., 1998) using the MIXED procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Treatment, time, and the interaction of the two variables were included in statistical models as fixed effects and starting measurements were included as covariates. Pen nested within treatment was considered random for growth, intake, blood metabolites, and rumen fermentation characteristic models. *In vitro* cellulose disappearance and gas production were analyzed as a single measurement by heifer at the conclusion of each period. Means reported for cellulose disappearance and gas production are from the highest dilution with a significant difference for the response variable. Variance-covariance matrix structures were evaluated for each model using simple, first order auto-regressive, compound symmetry, and unstructured covariance structures and were selected for each model based on the lowest Bayesian information criterion fit statistic. Orthogonal contrasts tested linear and quadratic responses to increasing grain inclusion during the treatment period only. Least squares means and standard errors of the mean are reported on a per heifer basis and mean differences were separated using the Tukey-Kramer method. When interactions of fixed effects were significant, the SLICE option was used to identify specific significant

effects. Statistical differences were considered significant at $P \leq 0.05$ and trends at $0.10 \geq P > 0.05$..

4.4 Results and Discussion

4.4.1 Heifer Weight and Skeletal Growth

Weights and ADG responses are presented in Table 4.2. Increasing concentrate inclusion from 40 to 80% of the dietary DM resulted in a greater BW overall ($P < 0.01$), as average BW increased 5.5% from 60:40 to 40:60 and 4.2% from 40:60 to 20:80. During the treatment period, average overall BW linearly increased as concentrate inclusion increased in the diet ($P < 0.01$), averaging 150.4, 157.0, and 163.6 kg for heifers fed 60:40, 40:60, and 20:80, respectively. A treatment×time interaction was also observed during the treatment period ($P < 0.01$), as BW were similar among treatments on d 14, but were greatest for heifers fed 20:80 on d 28, 42, and 56 of the study (Figure 4.1). Following a diet change, BW advantages were maintained for heifers fed 20:80 during the treatment period, as heifers were 9.2 and 22.2 kg heavier than heifers fed 40:60 ($P = 0.02$) and 60:40 ($P < 0.01$), respectively, at the conclusion of the study. However, total BW gain responses during each period of the study exhibited a treatment×time interaction ($P < 0.01$); total BW gain from d 0 to 56 was 77.1% and 29.8% greater for heifers fed 20:80 compared to 60:40 and 40:60, respectively, whereas total BW gain was 9.3 to 10.1% lower for heifers previously fed 20:80 compared with 60:40 and 40:60, respectively. Average daily gain was improved for heifers fed 20:80 during the treatment period compared with heifers fed 40:60 or 60:40 (1.04, 0.85, and 0.62, respectively). During the grower period, however, ADG tended to improve for

heifers previously fed 40:60 or 60:40 compared to heifers fed 20:80 (0.86, 0.88, and 0.78 kg/d, respectively). Following the switch from treatment diets to the common diet, a treatment×time interaction was apparent as ADG was significantly reduced by 47.7% (from 1.3 to 0.7 kg/d) for heifers previous fed 20:80 ($P < 0.01$), whereas ADG tended to increase 18.9% (from 0.7 to 0.9 kg/d) for heifers previously fed 60:40 ($P = 0.07$). Additionally, ADG were 27.3% and 37.1% greater 14 d ($P = 0.02$) and 28 d ($P < 0.01$) following a diet change for heifers previously fed 60:40 and 40:60 compared to 20:80 (Figure 4.2). However, ADG were similar among treatments from d 85 to the conclusion of the study ($P > 0.05$). Differences in growth rates following an abrupt diet change were likely driven by a reduction in DM intake observed for heifers receiving 20:80 during the first 28 d following the diet switch (discussed below), as well as an abrupt disruption in rumen fermentation shifting from a high concentrate to a high forage ration. Ending weights observed in the current study agree with those reported by Heinrichs and Losinger (1998) for heifers between 7.5 and 8.5 mo of age. Anderson et al. (2009) reported weights that were 26.0 kg heavier than those observed in the current study for heifers that were 1 mo younger; however, ADG were 32% greater than those observed in the current study which would explain the discrepancy in BW. When Jones et al. (1985) fed beef feeder steers low- or high-forage diets (30:70 vs 50:50 F:C ratio), live BW were 15 kg heavier at slaughter for steers fed a low-forage diet, though the difference was not statistically significant as steers were scheduled to be harvested at similar BW. Zanton and Heinrichs (2009b) reviewed the effects of altering dietary F:C ratios for growing dairy heifers, acknowledging that N utilization improves and N retention in tissues increases with increasing concentrate inclusion in the diet, regardless of total N intake.

Taken together, these observations suggest that increased BW in the current study may be due to increased energy and protein retention, agreeing with previous literature noting improved metabolic efficiency with increasing concentrate inclusion in the diets of growing cattle (Reynolds et al., 1991; Huntington et al., 1996)

Frame growth exhibited similar responses to those observed for BW and ADG (Table 4.3). Hip heights (Figure 4.3), WH (Figure 4.4), HGC, HW, and BCS increased with increasing concentrate inclusion over the entire study ($P < 0.01$); however, overall responses can be mostly attributed to linear increases observed during the treatment period for skeletal measurements. Overall growth in HH ($P < 0.01$) and HGC ($P < 0.01$) from d 0 to 112 was significantly greater for heifers fed 20:80 and 40:60 compared with 60:40. Similarly, WH ($P < 0.01$) and HW ($P = 0.02$) growth overall from d 0 to 112 was higher for heifers fed 20:80 compared with 40:60 and 60:40. Linear increases in overall daily growth rates were observed for HH ($P < 0.01$), WH ($P < 0.01$), and HGC ($P = 0.03$) as concentrate inclusion was greater in the treatment period. Though heifers fed 20:80 exhibited the greatest amount of growth for all parameters overall, daily growth rates for HH, WH, HGC, and HW were similar among treatments when fed a common diet. Similar to responses in ADG immediately following the switch to a common diet, total monthly gain in HH was significantly reduced 34.0% from d 56 (4.7 cm) to d 84 (3.1 cm) for heifers previously fed 20:80 compared to more consistent growth observed in heifers previously fed 40:60 and 60:40 (Figure 4.5). Conversely, monthly gain in WH was similar between heifers previously fed 20:80 and 40:60 from d 56 to d 84, but increased 72.0% for heifers previously fed 60:40 following a diet switch (2.4 to 4.1 cm; Figure 4.6). Monthly gain in HGC was similar between treatments following a diet switch (Figure

4.7). Monthly gain in HW was much more variable throughout the study compared to HH, WH, and HGC, but monthly growth for heifers previously fed 40:60 was significantly reduced 39.5% following a diet switch (2.1 to 1.3 cm; Figure 4.8). Overall, frame growth rates from d 0 to d 28 was greatest for heifers fed 20:80 during the treatment period compared to heifers fed 60:40 but, in general, declined over time compared to heifers fed 40:60 or 60:40. Heinrichs and Losinger (1998) reported average BW and WH for Holstein heifers in the U.S. of 214.9 kg and 107.6 cm, respectively, at 7.5 mo of age. Ending WH in the current study ranged from 3.8% to 6.5% higher than averages reported by Heinrichs and Losinger (1998) for 7.5 mo old heifers, and WH, HH, and HW were above median reported values for Holstein heifers of the same age according to Jones and Heinrichs (2013). Skeletal measurements observed in the current study were similar to those of Gabler and Heinrichs (2003a) for heifers fed diets with increasing CP:ME ratios from 4 mo to 8.5 mo of age. As heifers fed 20:80 were tallest at the hip and withers at the conclusion of the study compared to heifers fed 60:40, earlier breeding would be possible when feeding greater amounts of concentrate before puberty. Heinrichs et al. (1992) evaluated more than 2500 BW and WH measurements and reported close, significant quadratic and cubic relationships of BW with WH and vice versa ($R^2 > 0.92$). Additionally, the onset of puberty in heifers is closely associated with BW and body composition. Lammers et al. (1999a) reported heifers were 32 d younger with similar BW at puberty (determined by progesterone concentrations > 1 ng/mL) when fed for 1.0 kg/d ADG compared to 0.7 kg/d ADG from 19 to 39 wk of age. Withers height growth rates also increased 12% when increasing ADG from 0.7 to 1.0 kg/d (Lammers et al., 1999b). Other research groups have observed similar relationships of

accelerated growth rates with younger heifers at puberty where increased growth rates were achieved by feeding approximately 68% (Petitclerc et al., 1983) to 80% concentrate (Gardner et al., 1977) in the diet on a DM basis. Since frame growth closely follows BW and Holstein heifers typically reach 75% of mature withers height by 12 mo of age (Kertz et al., 1998), achieving greater frame growth rates prior to puberty could result in earlier breeding and younger heifers at first calving. Feeding heifers 60:40 reduced BCS 7.2% from d 0 to d 56, whereas feeding 40:60 maintained BCS and 20:80 increased BCS 4.0% from d 0 to d 56 ($P < 0.01$). Following a diet change, BCS for heifers previously fed 20:80 or 40:60 diets were, on average, 3.9% lower at the conclusion of the study than those observed on d 56, whereas heifers previously fed 60:40 exhibited BCS that were 3.1% greater at the conclusion of the study than those observed on d 56 ($P < 0.01$). Davis Rincker et al. (2008) observed increased BCS and carcass adiposity in heifers fed high-energy compared to low-energy diets from 11 to 23 wk of age. Using comparative slaughter, the authors also observed increased 12th-rib and perirenal fat deposition in heifers fed high-energy diets (Davis Rincker et al., 2008b). Reynolds et al. (1991) observed that when beef heifers were fed for constant ME intake, whole body heat production was lower and tissue energy retention was greater for heifers fed 75% concentrate versus heifers fed 25% concentrate, illustrating the importance of dietary energy source consideration in growing heifer diets. As BCS increased linearly with increasing inclusion of concentrate, it is likely that a portion of the observed increases in BW were partially due to increased subcutaneous fat deposition in addition to frame growth during the treatment period. While increased growth rates for young replacement heifers often results in reduced ages at first calving and increased milk production in the

first lactation (Soberon and Van Amburgh, 2013), excess energy deposited as fat post-weaning but before puberty can alter mammary gland development in favor of increased mammary fat pad deposition and similar or reduced parenchymal tissue (Capuco et al., 1995; Petitclerc et al., 1999). Despite reduced parenchymal tissue observed in 175 kg heifers fed for 950 g/d ADG compared to 725 g/d ADG on corn silage-based diets in the study by Capuco et al. (1995), first lactation milk production was similar between rates of gain. When followed through the first (Zanton and Heinrichs, 2007) and second (Zanton and Heinrichs, 2009b) lactations, 4% fat-corrected milk production (305-d mature equivalent) tended to be greater for cows previously limit-fed low- compared to high-forage diets as weaned, prepubertal heifers. Impaired mammogenesis appears to be related more to increased BCS at breeding (Silva et al., 2002) and low CP:ME ratio in the prepubertal diet (Whitlock et al., 2002) than overall ADG to puberty. However, mammary gland composition was not determined in the current study and information is limited with respect to the effects of low- vs. high-forage feeding to growing heifers for unrestricted intake and ADG on future milk production. Additionally, BCS observed in the current study were not excessive, ranging on average from 2.5 to 2.8 throughout the study.

4.4.2 Dry Matter and Nutrient Intake

Average daily DMI increased with increasing concentrate inclusion ($P < 0.01$), averaging 5.4, 5.6, and 6.0 kg/d for heifers fed 60:40, 40:60, and 20:80, respectively, from d 0 to d 112 (Table 4.2). However, when analyzed by study period, differences in DMI were only observed during the treatment period ($P < 0.01$; Figure 4.9). Following a

switch to a common diet, daily DMI averaged 6.5, 6.4, and 6.2 kg/d for heifers previously fed 60:40, 40:60, or 20:80, respectively ($P = 0.14$). Differences in daily intake of ME, CP, and NDF followed the same responses as DMI, with the exception of fNDF intake decreasing with increasing concentrate inclusion, both overall and linearly during the treatment period as designed ($P < 0.01$). Intake expressed as a percent of BW was similar across treatments overall, averaging 3.0% of BW ($P = 0.18$). During the treatment period, average DMI as a percent of BW (Figure 4.10) increased linearly with increasing concentrate inclusion from 2.7 to 3.3% of BW ($P < 0.01$). Heifers fed 60:40 increased DMI from 2.7% to 2.8% of BW ($P < 0.01$) during the treatment period, whereas heifers fed 20:80 increased from 3.1 to 3.4% of BW ($P < 0.01$). However, following the switch to the common grower diet, average DMI decreased from 3.4% of BW on d 56 to 2.6% of BW on d 70 for heifers previously fed 20:80 ($P < 0.01$) while average DMI increased from 2.8% to 3.1% of BW for heifers previously fed 60:40 from d 56 to d 70 ($P < 0.01$). Interestingly, average DMI as a percent of BW for heifers fed 40:60 was constant across both periods of the study and did not differ over time. Energy intakes, expressed as Mcal ME/100 kg of BW, linearly increased as concentrate inclusion increased in the treatment diets (6.8 to 9.2 Mcal ME/100 kg of BW; $P < 0.01$); however, ME intakes were greatest for heifers previously fed 60:40 and least for 20:80 ($P < 0.01$). Similar to DMI, heifers fed 40:60 maintained constant ME intake across both periods, though ME intake significantly declined immediately following a diet change as anticipated (8.0 to 7.1 Mcal ME/100 kg of BW; $P < 0.01$). Despite CP intakes (kg/d) being similar among treatments during the grower period, when expressed as a percent of BW, CP intake was greatest for heifers previously fed 60:40 compared to 40:60 ($P = 0.01$) and 20:80 ($P < 0.01$). Total

NDF intake as a percent of BW did not differ among treatments from d 0 to d 112 and averaged 1.3% of BW; however, differences were detected within each period (Figure 4.11). As concentrate inclusion in the diet increased during the treatment period, total NDF intake increased linearly from 1.0% of BW for heifers fed 60:40 to 1.2% of BW for heifers fed 20:80 ($P < 0.01$; Figure 4.11). Conversely, during the grower period, total NDF intake was greatest for heifers previously fed 60:40 and declined with previous concentrate inclusion level (1.6%, 1.5%, and 1.4% of BW for 60:40, 40:60, and 20:80, respectively; $P < 0.01$). Forage NDF intakes as a percent of BW were greatest for heifers fed 60:40 throughout the study ($P < 0.01$; Figure 4.12). Differences in fNDF intake were expected during the treatment period given the design of the study; however, when switched to a common diet, heifers previously fed 60:40 consumed significantly more fNDF as a percent of BW (1.0%) compared to 40:60 (0.9%; $P = 0.01$) and 20:80 (0.8%; $P < 0.01$).

Intakes observed in the current study disagree with those reported by Hoffman et al. (2008) for pen-fed Holstein heifers. Dry matter intakes were 10.5% lower and 21.6% higher for heifers fed 60:40 and 20:80, respectively, compared with reported values in Hoffman et al. (2008) for heifers fed diets similar in energy and NDF content. Reasons for disagreement in values are unclear, as those authors did not report diet ingredient composition. However, it is common to feed weaned replacement heifers diets containing corn silage and other ensiled forages, which have been shown to depress DMI compared to diets with higher DM content fed to mature cows (Lahr et al., 1983) or growing heifers (Thomas, 1961; Dennis et al., 2012). As a percent of BW, DMI observed in the current study agree with those observed by Davis Rincker et al. (2008) for 11 to 23

wk old heifers. The authors reported greater DMI throughout the 12 wk trial for heifers fed a high-energy diet (2.82 Mcal ME/kg of DM) compared with a low-energy diet (2.32 Mcal ME/kg of DM), which also translated to increased BW and skeletal growth.

Increased ME and CP intake for heifers fed 20:80 during the treatment period likely explains improved growth observed early in the study, as N utilization increases with increasing energy intake above maintenance (Garrett, 1980). However, once N utilization is maximized, excess energy can be stored as adipose tissue, which has been illustrated by Petitclerc et al. (1984) for dairy heifers fed for 1.0 kg/d compared to 0.7 kg/d of ADG exhibited 19.4% more carcass fat at 340 kg of BW. Increases in BCS during the treatment period for heifers fed 20:80 may reflect increased adiposity due to increased ME intake above requirements for protein synthesis. Hill et al. (2013) reported optimal CP:ME ratios for weaned heifers from 4 mo of age to breeding ranging from 61 to 65 g of CP/Mcal of ME. During the treatment period, CP:ME ratios averaged 72.5, 70.0, and 68.7 g of CP/Mcal of ME for 60:40, 40:60, and 20:80, respectively. Though all treatments supplied CP in excess of reported optimal values, it appears that as CP:ME increased in the current study, ADG and skeletal growth responded negatively. Pirlo et al. (1997) reported increased ADG from 100 to 200 kg of BW when TDN and CP were 110% of NRC requirements, though only significant effects of dietary TDN were observed when evaluating diets for prepubertal Italian Friesian heifers. The authors also observed that decreasing TDN and increasing CP did not result in acceptable growth rates (Pirlo et al., 1997), similar to results of the current study for heifers fed 60:40 during the treatment period and illustrating the importance of satisfying energy requirements to achieve targeted ADG in heifers. Energy availability in the 60:40 diet may have been

restricted despite exceeding NRC requirements, as a greater proportion of dietary ME was provided by forage fiber compared to concentrate. As energetic efficiency of fiber digestion is less than that of starch (VandeHaar and St-Pierre, 2006), corresponding responses in growth may reflect an inability of dairy heifers at this age to utilize forages in an appreciable capacity.

Consumption of total and forage NDF for heifers in the current study also disagree with previous work predicting intake in growing heifers. Voluntary DMI in lactating cattle is mostly controlled by physical and chemical factors (Allen, 2000), and is highly dependent on the proportion of NDF in the diet. Hoffman et al. (2008) reported that total NDF intake as a percent of BW was near-constant at 1.0% of BW from weaning to calving for Holstein heifers. However, heifers consumed between 1.0% and 1.6% of BW as total NDF throughout the current study, with greater NDF intakes observed during the grower period with a higher forage diet. Total NDF was consistent across treatment diets (approximately 37.5% of the dietary DM), suggesting that when fed a lower forage diet, total NDF is not physically restrictive on DMI for young dairy heifers. As there is less potential for small particle entrapment in the rumen due to shorter particle length in the fiber mat fraction, feeding lower forage diets often results in increased passage rates and intakes of higher NDF non-forage fiber sources (Grant, 1997). However, considering forage NDF intake was 0.6% of BW for heifers fed 60:40 and total DMI was lowest for that group during the treatment period, it stands to reason that forage NDF, and not total NDF, is a more accurate predictor of intake at this age. This may also suggest that the ability of heifers at this age to digest forage NDF is limited. While Moody et al. (2007) reported a tendency for improved apparent DM digestibility as Holstein heifers aged from

6 to 12 mo of age fed corn silage-based diets, apparent NDF digestibility values were similar regardless of age. However, as finely processed corn silage was the only forage source used and intake was restricted to approximately 80 g of DM per kg of $BW^{0.75}$ in the previous study, reduced particle size and DM intakes less than 2.0% of BW would potentially confound any effects of age related to forage NDF digestibility. In the current study, dry hay was the sole forage source and was moderately processed to reduce particle size prior to feed delivery. Compared to finely chopped corn silage, forage used in the current study would have required more mastication by heifers to reduce particle size, as particle size reduction is required for flow from the rumen and larger particles can restrict voluntary DMI (Allen, 1996). Van Soest (1996) also reported that gut capacity is isometrically related to body size in ruminants, and taken together with theoretically increased time to reduce particle size for heifers fed higher proportions of hay could potentially explain intake responses in the current study.

4.4.3 Feed and Nutrient Efficiencies

Feed efficiency was significantly improved with increasing concentrate inclusion during the treatment period (Table 4.2); however, following the diet change, G:F was similar among treatments. Nutrient efficiencies for ME, CP, and NDF followed the same linear responses as corresponding nutrient intakes during the treatment period. While feed and nutrient efficiencies were similar among treatments during the common feeding period, a treatment×time interaction was observed. Heifers fed 40:60 during the treatment period were more efficient at converting nutrients to gain 14 (P = 0.10) and 28 d (P = 0.04) following a diet change compared to heifers fed 20:80 (0.163 and 0.166 for

60:40; 0.131 and 0.125 for 20:80 on d 56 and d 70, respectively). Additionally, G:F was reduced 33.7% (0.197 to 0.131) for heifers previously fed 20:80 from d 56 to d 70 of the study ($P < 0.01$) but G:F were similar on d 70 for heifers previously fed 60:40 and 40:60 to those on d 56 (Figure 4.13). As BW gain during the common feeding period decreased linearly for heifers previously fed increasing levels of concentrate and DMI did not respond similarly, higher proportions of concentrate likely negatively affected the ability of growing heifers to transition to higher forage diets, and heifers fed greater amounts of forage were better adapted to utilize forages later in the growing period.

Growth and intake responses for heifers fed 60:40 appear to be compensatory in nature following the treatment period, as ME and CP intakes increased by 52.1% and 41.6%, respectively, while on the common diet. This is in contrast to 20:80 heifers reducing ME and CP intake by 2.5% and 8.0%, respectively, which was likely a function of depressed intake following the diet change and maintained throughout the common feeding period. Additionally, G:F was reduced, but not significantly different, for heifers fed 60:40 between treatment and common diet feeding periods ($P = 0.43$), whereas G:F for heifers fed 40:60 and 20:80 were significantly lower in the common feeding period compared to the treatment period ($P < 0.01$). Net efficiency of fiber utilization, whether from forage or non-forage sources, is generally lower than that of starch (VandeHaar and St-Pierre, 2006), which supports observations for G:F in the current study with increasing inclusion of concentrate in the diet.

4.4.4 Feed Costs and Cost per Gain

Feed costs per kg of DMI averaged $0.24, $0.27, and $0.29 for heifers fed 60:40, 40:60, and 20:80, respectively, during the treatment period. Total feed costs increased as concentrate increased in the treatment diets ($P < 0.01$), ranging from $141.74 to $170.68 per heifer for 112 d (Table 4.3). When calculating feed costs using 5 yr commodity averages, feed costs were 14.2 to 15.5% lower than costs incurred during the current study. However, total feed costs still increased as concentrate inclusion increased during the treatment period ($P < 0.01$). Costs per head increased for all treatments over time ($P < 0.01$), as well as the order of magnitude of differences between treatments. Daily feed costs per hd were 21.9% and 44.7% greater for 20:80 than 40:60 and 60:40, respectively ($P < 0.01$), on d 14 of the trial and subsequently increased with increased DMI. On d 56 prior to switching to a common diet, feed costs per hd were 68.1% and 32.5% greater for 20:80 than 60:40 and 40:60 ($P < 0.01$), respectively. Following the diet switch, feed cost per kg of DMI averaged $0.23 and feed costs per hd were similar among treatments.

Feed costs per kg of ADG (C:ADG) were lowest for 40:60 heifers over the duration of the study compared to heifers fed 60:40 ($P = 0.04$), though were similar to feed costs incurred per kg of gain in 20:80 heifers ($P = 0.13$). When heifers were fed 40:60 or 20:80 during the treatment period, C:ADG savings were $0.51 ($P = 0.02$) per kg of ADG compared to heifers fed 60:40. Interestingly, despite lack of overall treatment effects when heifers were fed a common diet, a treatment×time interaction was observed ($P = 0.05$). For each kg of ADG, heifers previously fed 20:80 were $0.72 more expensive to feed than heifers fed 60:40 on d 28 following a switch to a common diet ($2.18 vs. $1.46 per kg of ADG). However, at the conclusion of the trial, C:ADG tended

to be $0.58 greater for heifers previously fed 60:40 compared to 20:80 ($2.58 vs. $2.00 per kg of ADG; $P = 0.08$). Overall C:ADG tended to be affected by treatment ($P = 0.10$), and heifers fed 20:80 were $0.29 more expensive per kg of ADG compared to heifers fed 40:60. However, when accounting for 5 yr average commodity prices, overall C:ADG were similar among treatments ($P = 0.16$), ranging from $1.42 to $1.64 per kg of ADG. Though heavily dependent on forage quality and energy content, increased concentrate consumption typically results in increased income over feed costs for lactating cows (Smith, 1976). Similarly, beef steers allowed free-choice consumption of feed components compared to a TMR were less expensive to feed per kg of DMI and Mcal of ME intake when steers chose diets with higher proportions of concentrates than forages (Atwood et al., 2001). As heifers fed increasing levels of concentrate would likely reach puberty and breeding weight sooner than heifers fed lower concentrate diets, there is potential to reduce cost per kg of ADG by improving FE and reducing days on feed.

4.4.5 Blood Metabolites

Overall, blood glucose concentrations increased linearly with increasing inclusion of concentrate during the treatment period (Figure 4.14; $P = 0.03$). Most of the response can be attributed to the treatment feeding period, as blood glucose was elevated for heifers fed 20:80 compared to heifers fed 40:60 ($P = 0.07$) and 60:40 ($P < 0.01$). Following a diet change, a treatment×time interaction was observed as glucose concentrations tended to be greater for heifers previously fed 40:60 compared to 20:80 on d 84, whereas glucose was greatest for heifers previously fed 60:40 on d 112 compared to

40:60 ($P < 0.01$) and 20:80 ($P = 0.02$). Additionally, blood glucose significantly increased 25.7% and 39.5% from the end of the treatment period for heifers previously fed 60:40 on d 84 and d 112, respectively. Increased concentrations of glucose relative to the treatment period for heifers previously fed 60:40 may indicate increased efficiency of energy utilization from a higher forage diet. Huntington (1989) observed beef steers fed either alfalfa hay or a high-concentrate diet at similar ME intakes and found arterial concentrations of glucose were similar between treatments, which is in contrast to the current study during the common feeding period. Schoonmaker et al. (2003) reported similar serum glucose concentrations when beef steers (181 d of age) were fed all-concentrate or all-fiber diets *ad libitum*, also in contrast to the current study. However, post-prandial serum insulin concentrations were significantly elevated for steers fed all-concentrate compared to all-fiber, suggesting higher glucose utilization by peripheral tissues in response to greater glucose supply from a high concentrate diet (Schoonmaker et al., 2003). Glucose concentrations are highly related to propionate metabolism from rumen fermentation, and increased glucose concentrations observed for heifers fed 60:40 may be related to the increase in propionate observed from d 56 to d 112 ($P = 0.04$; discussed below).

Plasma urea N concentrations exhibited a treatment×time effect where PUN increased with increasing concentrate inclusion on d 28 ($P = 0.01$) and d 56 ($P = 0.04$), but were similar following a diet change (Figure 4.15). Increased PUN was likely a result of increased DMI with increasing inclusion of concentrate, as CP intake was significantly greater for heifers fed 20:80 compared to 60:40 during the treatment period (Table 4.3). Concentrations ranged from 10.5 to 13.1 mg/dL of urea N across all

treatments during the treatment period, which is indicative of average levels expressed by growing cattle at maximized growth rates (Byers and Moxon, 1980). Following a diet change, PUN was significantly lower on d 84 compared to d 56, decreasing from 11.1 to 9.6 mg/dL on average. This response is likely reflective of a reduction in dietary CP content compared to treatment diets, as well as reduced DMI for heifers previously fed higher inclusions of concentrate.

4.4.6 Rumen Fermentation Characteristics

As concentrate inclusion increased in the diet during the treatment period, rumen fermentation was altered in favor of lower rumen pH, higher concentrations of NH3, and increased molar proportions of propionate and butyrate (Table 4.5). Rumen pH decreased linearly ($P < 0.01$) as concentrate inclusion increased in the diet during the treatment period; however, pH, total VFA, molar proportions of individual VFA, and rumen NH3 was similar among treatments when heifers were fed a common diet during the grower period. A treatment×time interaction was observed for rumen pH during the treatment period, as heifers fed 60:40 tended to have higher pH than heifers fed 20:80 on d 28 ($P = 0.08$), and significantly higher pH than 40:60 ($P < 0.01$) and 20:80 ($P < 0.01$) on d 56 (Figure 4.16). During the treatment period, a treatment×time interaction was observed for total VFA concentrations and all individual VFA molar proportions (listed in Table 4.5), with the exception of total isoacids. At the end of the treatment period, total VFA concentrations were greatest for heifers fed 20:80 and 40:60 compared to heifers fed 60:40 (118.6, 107.4, and 74.4 mM, respectively; $P < 0.01$). However, once switched to a common diet in the grower period, total VFA concentrations were similar

among treatments on d 84 and d 112 (Figure 4.17). Molar proportions of acetate decreased linearly with increasing inclusion of concentrate in the diet during the treatment period ($P < 0.01$), with heifers fed 60:40 exhibiting 69.9% of total VFA as acetate compared to 58.8% for heifers fed 20:80 (Figure 4.18). In contrast, heifers fed 20:80 had significantly greater proportions of propionate ($P < 0.01$; Figure 4.19), butyrate ($P < 0.01$; Figure 4.20), and valerate ($P < 0.01$) compared to heifers fed 60:40 during the treatment period. Additionally, molar proportions of propionate ($P < 0.01$) and valerate ($P < 0.01$) were reduced for heifers fed 40:60 compared to heifers fed 20:80 during the treatment period. As expected, acetate:propionate ratio (A:P) decreased with increasing concentrate inclusion during the treatment period (Figure 4.21). Reduced rumen pH and altered VFA profiles with increasing inclusion of concentrate agree with findings by Reis and Combs (2000) in lactating dairy cows and Lascano and Heinrichs (2009) in dairy heifers. Reis and Combs (2000) observed that though total VFA was similar among cows supplemented with 0, 5, or 10 kg of corn-based concentrate on pasture, concentrations of propionate and butyrate increased and A:P decreased with increased concentrate supplementation. Similarly, Lascano and Heinrichs (2009) found as concentrate increased from 20 to 60% of the diet on a DM basis in a corn silage-based diet, molar proportions of acetate were linearly decreased and proportions of propionate were linearly increased. Reduced rumen pH and greater VFA concentrations are associated with increased absorption of VFA across the rumen epithelium (Dijkstra et al., 1993; Gäbel et al., 2002). Additionally, altering rumen fermentation in favor of propionate production increases gluconeogenic potential in cattle, which is related to increases in prepubertal growth (McCartor et al., 1979) and milk production (Seymour et al., 2005).

As pH was reduced during the treatment period for heifers fed 20:80, growth and efficiency responses were likely linked to improved availability and absorption of VFA from rumen fermentation. It has also been extensively shown that propionate, along with butyrate, has proliferative effects on rumen tissue development in calves (Baldwin et al., 2004). As performance for heifers fed 60:40 was reduced and likely related to impaired utilization of the diet, rumen development at this age may be incomplete and diets should include higher proportions of concentrate (up to 60% of the diet on a DM basis) to optimize growth.

Rumen NH3 concentrations linearly increased with increasing concentrate inclusion in the diet from 15.8 mg/dL for heifers fed 60:40 to 19.0 mg/dL for heifers fed 20:80 ($P = 0.05$). Similar to other fermentation characteristics, NH3 concentrations were similar among treatments following a switch to a common diet. However, a treatment×time interaction was observed as heifers previously fed 20:80 had rumen NH3 concentrations decline 10.3 mg/dL from d 56 to d 84, whereas heifers previously fed 60:40 and 40:60 declined 5.5 mg/dL and 4.2 mg/dL, respectively from d 56 to 84 (Figure 4.22). This result is likely related to the decrease in dietary CP from the treatment to the grower period, as less N was available to the rumen. Additionally, the more pronounced reduction in rumen NH3 for heifers previously fed 20:80 may be due to differences in N availability in the rumen going from the treatment to the grower period. Differences in rumen NH3 may also be related to synchrony of carbohydrate and N substrates in the rumen. Hristov et al. (2005) illustrated that as carbohydrate degradation rates increase in the rumen, rumen NH3 concentrations are reduced as more bacterial N is synthesized in synchrony with available carbohydrate sources. However, rumen NH3 concentrations for

heifers fed 20:80 were elevated compared to heifers fed lower proportions of concentrate. Most of this response can likely be attributed to increased CP intake, but potential differences in carbohydrate supply to rumen bacteria cannot be overlooked. Greater than 40% of the 20:80 diet was comprised of a high-NDF complete feed source, which likely contained high concentrations of plant by-product NDF sources (such as soybean hulls, wheat middlings, and cottonseed hulls), whereas a greater proportion of the 40:60 and 60:40 diets came from corn. As NDF is degraded slower in the rumen compared to starch, asynchrony of carbohydrate and N sources could have occurred, resulting in increased rumen NH3 in addition to PUN for heifers fed 20:80 as excess N would recycle as urea. Despite similar NDF concentrations across treatment diets, differences in forage versus non-forage sources of NDF may have also influenced fermentation kinetics in the rumen (Firkins, 1997). However, as rumen NH3 increases with increased CP intake in ruminants (McIntyre, 1970; Slyter et al., 1979), these observations are likely a result of increased CP intake for heifers fed 20:80 during the treatment period.

An overall tendency for a treatment difference in i*n vitro* cellulose disappearance was observed ($P = 0.10$), as heifers fed 60:40 and 40:60 exhibited 10.0% and 11.5% greater cellulose digestion *in vitro*, respectively, compared to heifers fed 20:80. Disappearance was similar among diets on d 56 of the treatment period; however, cellulose disappearance was 11.4% greater for heifers previously fed 40:60 compared to heifers fed 20:80 on d 112 ($P = 0.04$). In contrast to cellulose disappearance, gas production on d 56 was 4.5 and 21 times greater for heifers fed 60:40 compared to heifers fed 40:60 ($P < 0.01$) and 20:80 ($P < 0.01$), respectively. A treatment×time interaction was observed from d 56 to d 112 ($P < 0.01$), as headspace gas production increased 0.5,

10.5, and 41 times for heifers previously fed 60:40, 40:60, and 20:80, respectively. These results suggest that rumen microbial populations during the treatment period were primarily acetogenic bacteria for heifers fed 60:40. Gas production as a result of glucose fermentation to acetate theoretically produces 33.3% and 50% more CO_2 than fermentation to butyrate and propionate, respectively (Beuvink and Spoelstra, 1992), suggesting that increases in gas production seen in heifers fed 60:40 during the treatment period are primarily due to increased acetogenic bacteria populations. Corresponding increases in the proportion of acetate produced for heifers fed 60:40 during the treatment period support this theory in the current study. Additionally, subsequent increases in cellulose disappearance and gas production from d 56 to d 112, as well as proportions of acetate for all heifers on a common diet suggest increased fermentation capacity for cellulose in the diet.

4.5 Summary and Conclusions

When evaluating growth performance of prepubertal dairy heifers provided diets with increasing proportions of concentrate, ADG, skeletal growth, and BCS increased as concentrate increased in the diet as expected. Dry matter intake, as well as ME, CP, and NDF intakes, increased as heifers consumed a larger proportion of concentrate in the diet. However, immediately following a rapid switch to a common grower diet at d 57, ADG and DMI were greatest for heifers previously fed a 40% concentrate diet. Heifers fed 60% concentrate during the treatment period had more consistent ADG and DMI throughout the study compared to heifers fed the most and least amount of concentrate. Intake of NDF were markedly higher than previously reported values for heifers between

4.5 and 8.5 mo of age, indicating that total NDF may not be an appropriate predictor of intake in young heifers. Feed efficiency and nutrient utilization were improved as the dietary concentrate levels increased. Feeding increasing proportions of concentrate resulted in a 10 to 22% increase in daily feed cost per heifer; however, when expressed relative to ADG, feed costs were reduced 10 to 15% for heifers fed 80% and 60% concentrate diets compared to 40% concentrate diets. When evaluated as a complete feeding program, feed cost savings per kg of gain were greatest for heifers fed 60% concentrate compared to heifers fed 40% concentrate under the conditions of this study. However, when accounting for 5 yr averages for commodity prices, costs per kg of gain were similar overall, indicating that feeding higher concentrate diets result in similar feed costs when they are evaluated on a gain basis and can be a cost effective method of feeding heifers. Rumen fermentation parameters were altered in favor of higher proportions of propionate and butyrate for heifers fed 80% concentrate diets during the treatment period, which was likely a factor in the increased circulating glucose concentrations. Reduced growth performance immediately following a diet change for heifers previously fed 80% concentrate may indicate negative effects of high concentrate diets on the ability to transition to high forage diets. Conversely, reduced performance for heifers fed 40% concentrate during the treatment period may be related to shifting rumen fermentation to acetate production in lieu of propionate and butyrate. However, heifers previously fed a high forage diet were less likely to experience reduced performance after switching to a diet approximately 30% more forage NDF. From the responses observed in the current study, feeding moderate to high proportions of concentrate to growing dairy heifers optimizes growth early in the grower period;

however, it appears in the current study that feeding a moderate concentrate diet optimized growth and feed efficiency while reducing feed costs compared to feeding low- or high-concentrate diets.

4.6 Acknowledgements

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Table 4.1. Ingredient composition and nutrient analysis (± s.d.) of treatment and grower diets.

Item	60:40[1]	40:60	20:80	Grower Diet
Ingredient, % of DM				
Alfalfa/orchardgrass hay	60.0	40.0	20.0	60.0
Grower feed[2]	0.0	21.0	43.2	17.9
Cracked corn	26.3	24.2	21.0	7.1
DDGS	5.3	5.3	5.3	11.4
SBM	8.4	9.5	10.5	3.6
Diet nutrient composition[3]				
DM	86.1 (0.3)	87.6 (0.9)	88.4 (0.5)	88.7 (0.3)
ME[4], Mcal/kg	2.51 (0.00)	2.63 (0.04)	2.67 (0.02)	2.46 (0.01)
NE_m[5], Mcal/kg	1.53 (0.0)	1.65 (0.03)	1.70 (0.02)	1.53 (0.01)
NE_g[6], Mcal/kg	0.93 (0.00)	1.03 (0.03)	1.09 (0.02)	0.92 (0.01)
TDN	66.4 (0.0)	69.3 (1.0)	70.1 (0.5)	65.3 (0.2)
CP	18.2 (0.0)	18.9 (0.5)	18.8 (0.2)	16.2 (0.0)
NDF	38.2 (0.0)	36.9 (0.1)	37.2 (1.9)	49.3 (0.8)
fNDF[7]	22.9	14.8	7.5	29.5
ADF	26.4 (0.1)	25.3 (0.1)	24.9 (0.9)	32.1 (0.0)
Ca	0.66 (0.00)	0.74 (0.03)	0.84 (0.02)	0.63 (0.01)
P	0.44 (0.02)	0.54 (0.01)	0.60 (0.02)	0.49 (0.00)

[1]Forage:concentrate ratio.
[2]Complete feed mix from CPC Commodities (Fountain Run, KY) with analysis of 2.64 Mcal/kg ME, 15.4% CP, 49.3% NDF, 65.7% TDN, 1.47% Ca, and 0.76% P on a DM basis and 29 g/ton monensin (as monensin-sodium) on an as-fed basis.
[3]All values given as a percent of DM unless otherwise stated.
[4]Calculated using the following equation: $ME = 1.01 \times [(0.04409 \times TDN) - 0.45]$.
[5]Calculated using the following equation: $NE_m = (1.37 \times ME) - (0.138 \times ME^2) + (0.0105 \times ME^3) - 1.12$.
[6]Calculated using the following equation: $NE_g = (1.42 \times ME) - (0.174 \times ME^2) + (0.0122 \times ME^3) - 1.65$.
[7]Forage NDF.

Table 4.2. Weight and skeletal growth responses of prepubertal dairy heifers fed increasing levels of concentrate during the treatment period then switched to a common diet.

					P-value[2]	
Item	60:40[1]	40:60	20:80	SEM	T	T×S
Body weight, kg						
d 0	132.9	133.6	133.1	2.74	--	--
d 56	167.8^{c}	181.2^{b}	194.9^{a}	2.73	< 0.01	--
d 112	216.4^{c}	229.4^{b}	238.6^{a}	2.74	< 0.01	--
ADG[3], kg/d						
d 0 to 56	0.62^{c}	0.85^{b}	1.10^{a}	0.036	< 0.01	0.64
d 57 to 112	0.88^{x}	0.87^{x}	0.78^{y}	0.029	0.06	< 0.01
d 0 to 112	0.75^{c}	0.86^{b}	0.94^{a}	0.024	< 0.01	< 0.01
Hip height, cm						
d 112	116.4^{c}	118.9^{b}	119.8^{a}	0.32	< 0.01	--
Monthly gain, d 0 to 56	3.0^{c}	3.9^{b}	4.7^{a}	0.23	< 0.01	0.92
Monthly gain, d 57 to 112	2.9	3.3	3.4	0.18	0.11	< 0.01
Withers height, cm						
d 112	111.7^{b,y}	112.6^{ab,x}	114.6^{a}	0.38	< 0.01	--
Monthly gain, d 0 to 56	2.5^{b}	3.0^{b}	4.1^{a}	0.25	< 0.01	< 0.01
Monthly gain, d 57 to 112	3.1	3.7	3.6	0.21	0.16	0.05
Hip width, cm						
d 112	34.0^{b}	34.4^{b}	35.3^{a}	0.21	< 0.01	--
Monthly gain, d 0 to 56	1.7^{b}	1.9^{ab}	2.2^{a}	0.13	0.06	< 0.01
Monthly gain, d 57 to 112	1.8	2.0	2.0	0.12	0.53	0.07
Heart girth, cm						
d 112	141.1^{b}	145.0^{a}	145.7^{a}	0.74	< 0.01	--
Monthly gain, d 0 to 56	6.7^{b}	8.2^{a}	8.6^{a}	0.47	0.03	0.21
Monthly gain, d 57 to 112	5.6	6.4	6.0	0.35	0.35	0.20
BCS[4], 1 to 5 scale						
d 0	2.68	2.68	2.67	0.030	--	--
d 56	2.49^{c}	2.67^{b}	2.78^{a}	0.030	< 0.01	--
d 112	2.56^{b}	2.57^{b}	2.67^{a}	0.030	0.02	--

[1]Forage:concentrate ratio.
[2]T = treatment effect; T×S = treatment×time interaction.
[3]Average daily gain.
[4]Body condition score.
[abc]Means with differing superscripts are significantly different at $P \leq 0.05$ level.
[xy]Means with differing superscripts tend to differ at $0.10 \geq P > 0.05$.

Table 4.3. Feed and nutrient intake responses of prepubertal dairy heifers fed increasing levels of concentrate during the treatment period then switched to a common diet.

					P-value[2]	
Item	60:40[1]	40:60	20:80	SEM	T	T×S
DM intake, kg/d						
d 0 to 56	4.23[c]	4.83[b]	5.75[a]	0.090	< 0.01	< 0.01
d 57 to 112	6.50	6.39	6.21	0.132	0.31	0.58
d 0 to 112	5.37[c]	5.61[b]	5.98[a]	0.075	< 0.01	< 0.01
DM intake, % of BW						
d 0 to 56	2.73[c]	2.96[b]	3.35[a]	0.044	< 0.01	0.07
d 57 to 112	3.26[a]	3.00[b]	2.80[c]	0.062	< 0.01	0.58
d 0 to 112	2.99	2.98	3.07	0.035	0.18	< 0.01
ME intake, Mcal/d						
d 0 to 56	10.6[c]	12.7[b]	15.8[a]	0.25	< 0.01	< 0.01
d 57 to 112	16.2	15.9	15.4	0.33	0.31	0.57
d 0 to 112	13.4[c]	14.3[b]	15.6[a]	0.19	< 0.01	< 0.01
CP intake, kg/d						
d 0 to 56	0.77[c]	0.91[b]	1.13[a]	0.017	< 0.01	< 0.01
d 57 to 112	1.09	1.07	1.04	0.022	0.31	0.58
d 0 to 112	0.93[c]	0.99[b]	1.09[a]	0.013	< 0.01	< 0.01
NDF intake, kg/d						
d 0 to 56	1.61[c]	1.77[b]	2.04[a]	0.033	< 0.01	< 0.01
d 57 to 112	3.17	3.11	3.02	0.064	0.31	0.58
d 0 to 112	2.39[b]	2.44[b]	2.53[a]	0.034	0.04	< 0.01
fNDF[3] intake, kg/d						
d 0 to 56	0.97[a]	0.71[b]	0.41[c]	0.016	< 0.01	< 0.01
d 57 to 112	1.93	1.89	1.84	0.039	0.31	0.58
d 0 to 112	1.43[a]	1.29[b]	1.11[c]	0.021	< 0.01	< 0.01
Feed efficiency[4]						
d 0 to 56	0.147[c]	0.178[b]	0.196[a]	0.008	< 0.01	0.55
d 57 to 112	0.136	0.139	0.128	0.005	0.31	0.04
d 0 to 112	0.142[b]	0.158[a]	0.161[a]	0.004	0.03	< 0.01

[1]Forage:concentrate ratio.
[2]T = treatment effect; T×S = treatment×time interaction.
[3]Forage NDF (% of DM).
[4]Feed efficiency expressed as kg of ADG per kg of daily DM intake.
[abc]Means with differing superscripts are significantly different at $P \leq 0.05$ level.

Table 4.4. Daily feed costs for heifers fed increasing levels of concentrate during the treatment period followed by a common diet.

Item	60:40[1]	40:60	20:80	SEM	*P*-value
Total feed cost					
Study costs[2]					
d 0 to 56	59.47[c]	72.68[b]	91.01[a]	1.434	< 0.01
d 57 to 112	82.31	81.29	79.65	1.626	0.53
d 0 to 112	141.74[a]	153.95[b]	170.68[a]	1.943	< 0.01
5 yr average costs[3]					
d 0 to 56	47.62[c]	59.74[b]	76.80[a]	1.181	< 0.01
d 57 to 112	72.02	71.13	69.70	1.423	0.53
d 0 to 112	119.61[c]	130.84[b]	146.49[a]	1.650	< 0.01
Daily feed cost per hd					
Study costs					
d 0 to 56	1.03[c]	1.29[b]	1.67[a]	0.024	< 0.01
d 57 to 112	1.48	1.45	1.41	0.030	0.31
d 0 to 112	1.26[c]	1.37[b]	1.54[a]	0.018	< 0.01
5 yr average costs					
d 0 to 56	0.83[c]	1.06[b]	1.41[a]	0.020	< 0.01
d 57 to 112	1.30	1.27	1.24	0.026	0.31
d 0 to 112	1.06[c]	1.17[b]	1.32[a]	0.16	< 0.01
Cost of gain[4]					
Study costs					
d 0 to 56	2.11[a]	1.60[b]	1.61[b]	0.135	0.03
d 57 to 112	1.81	1.75	1.91	0.114	0.62
d 0 to 112	1.96[a]	1.67[b]	1.76[ab]	0.088	0.10
5 yr average costs					
d 0 to 56	1.69[a]	1.31[b]	1.36[b]	0.109	0.06
d 57 to 112	1.58	1.53	1.67	0.100	0.62
d 0 to 112	1.64	1.42	1.51	0.074	0.16

[1]Forage:concentrate ratio.
[2]All values given in US dollars ($).
[3]Feed costs calculated using average commodity prices obtained from the USDA National Agricultural Statistics Service for 2007 to 2012 crop years.
[4]$/kg of average daily gain.
[abc]Means with differing superscripts are significantly different at $P \leq 0.05$ level.

Table 4.5. Rumen fermentation characteristics of prepubertal dairy heifers fed increasing levels of concentrate followed by a common diet.

					P-value	
Item	60:40[1]	40:60	20:80	SEM	T	T×S
Rumen pH						
d 0 to 56	6.63[a]	6.54[a]	6.33[b]	0.057	< 0.01	< 0.01
d 57 to 112	6.61	6.59	6.59	0.052	0.97	< 0.01
Rumen NH3, mg/dL						
d 0 to 56	15.8[y]	15.8[y]	19.0[x]	1.06	0.09	0.20
d 57 to 112	13.1	14.2	13.7	0.83	0.63	0.04
Total VFA[2], mM						
d 0 to 56	75.7[b]	92.2[a]	94.6[a]	4.09	0.01	0.03
d 57 to 112	81.0	77.2	79.1	3.89	0.74	< 0.01
Molar proportion of VFA[3]						
d 0 to 56						
Acetate	69.9[a]	67.2[b]	58.8[c]	0.90	< 0.01	< 0.01
Propionate	17.8[b]	18.6[b]	25.9[a]	0.86	< 0.01	< 0.01
Butyrate	8.6[b]	9.9[ab]	10.7[a]	0.47	0.03	< 0.01
Valerate	1.4[b]	1.5[b]	2.2[a]	0.09	< 0.01	< 0.01
Isoacids[4]	2.5	2.6	2.3	0.15	0.36	0.44
A:P[5]	4.18[a]	3.77[a]	2.48[b]	0.150	< 0.01	< 0.01
d 57 to 112						
Acetate	67.3	67.5	68.5	1.00	0.71	< 0.01
Propionate	19.2	19.2	17.8	0.63	0.31	< 0.01
Butyrate	10.0	10.1	10.1	0.42	0.96	< 0.01
Valerate	1.3	1.3	1.2	0.07	0.64	< 0.01
Isoacids	2.1	2.1	2.1	0.12	0.87	0.06
A:P	3.73	3.72	4.03	0.155	0.36	< 0.01
Cellulose disappearance[6]						
d 0	17.6	17.3	17.6	2.96	--	--
d 56	23.2	23.6	17.5	2.96	0.28	--
d 112	89.4[ab,x]	91.5[a]	82.1[b,y]	3.12	0.09	--
Gas production[7]						
d 0	1.4	1.5	1.7	0.29	--	--
d 56	2.2[a]	0.4[b]	0.1[b]	0.29	< 0.01	--
d 112	3.3[b]	4.6[a]	4.2[a]	0.29	< 0.01	--

[1]Forage:concentrate ratio.
[2]Volatile fatty acids.
[3]Molar proportion expressed as mol individual VFA/100 mol total VFA.
[4]Sum of isovalerate and isobutyrate molar proportions.
[5]Acetate:propionate ratio.
[6]Expressed as g/100 g of initial weight at 10^{-8} dilution.
[7]Expressed as total mL of headspace gas produced at 10^{-8} dilution.
[abc]Means with differing superscripts are significantly different at $P \leq 0.05$ level.
[xy]Means with differing superscripts tend to differ at $0.10 \geq P > 0.05$.

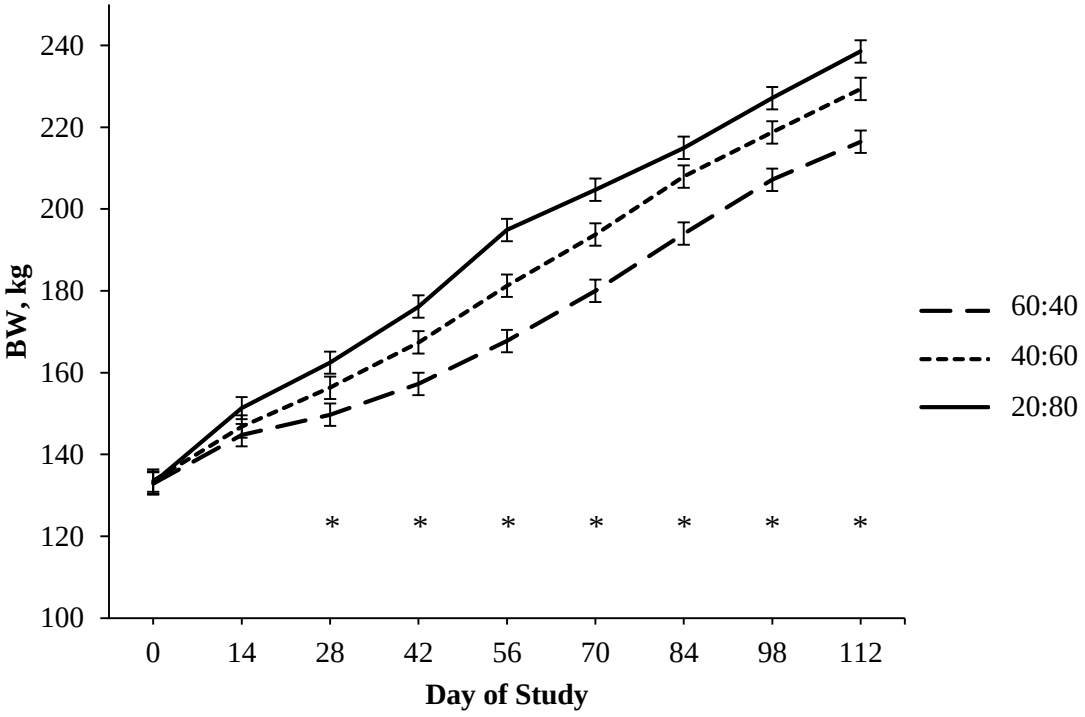

Figure 4.1. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on body weight (BW) over time. A treatment×time interaction was observed ($P < 0.01$) as BW diverged at d 28 of the study and was greatest for heifers fed 20:80 until the end of the study. 60:40 = 40% concentrate; 40:60 = 60% concentrate; 20:80 = 80% concentrate; *$P < 0.01$.

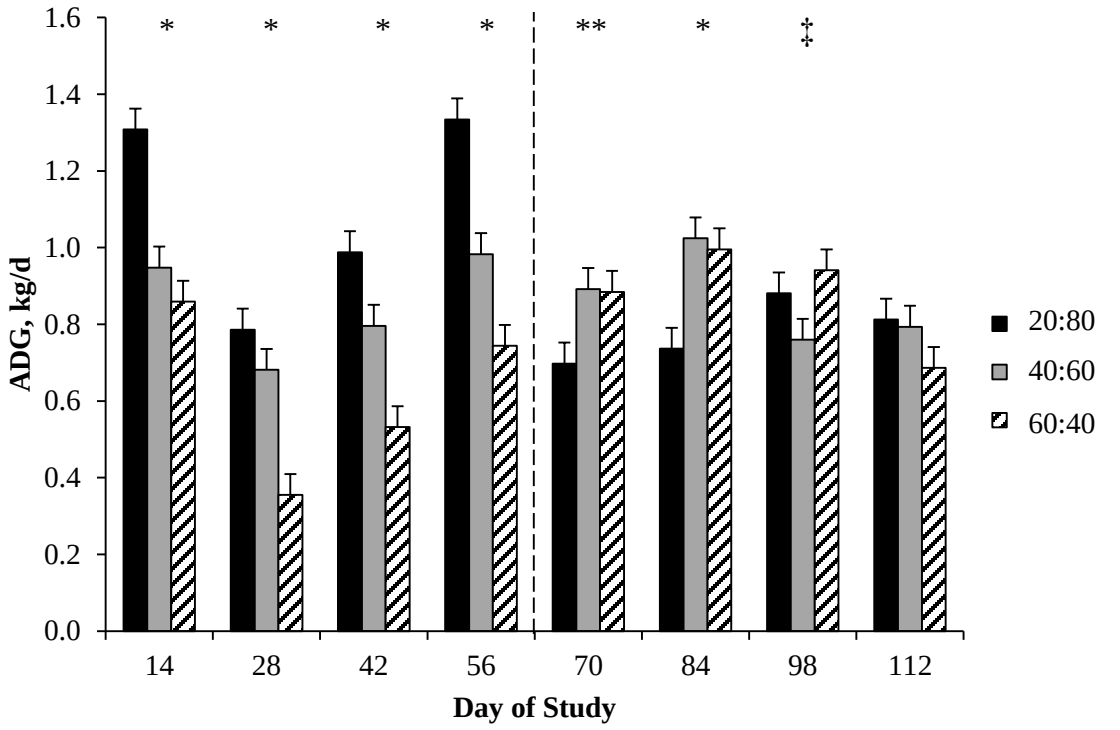

Figure 4.2. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on average daily gain (ADG) over time. Vertical dashed line indicates diet switch. A treatment×time interaction was observed ($P < 0.01$) as ADG was greatest for heifers fed 20:80 during the treatment period but least on d 70 ($P = 0.02$) and 84 ($P < 0.01$) following a diet switch compared to heifers previously fed 40:60 and 60:40. 60:40 = 40% concentrate; 40:60 = 60% concentrate; 20:80 = 80% concentrate; ‡$0.10 \leq P < 0.05$; **$P \leq 0.05$; *$P < 0.01$.

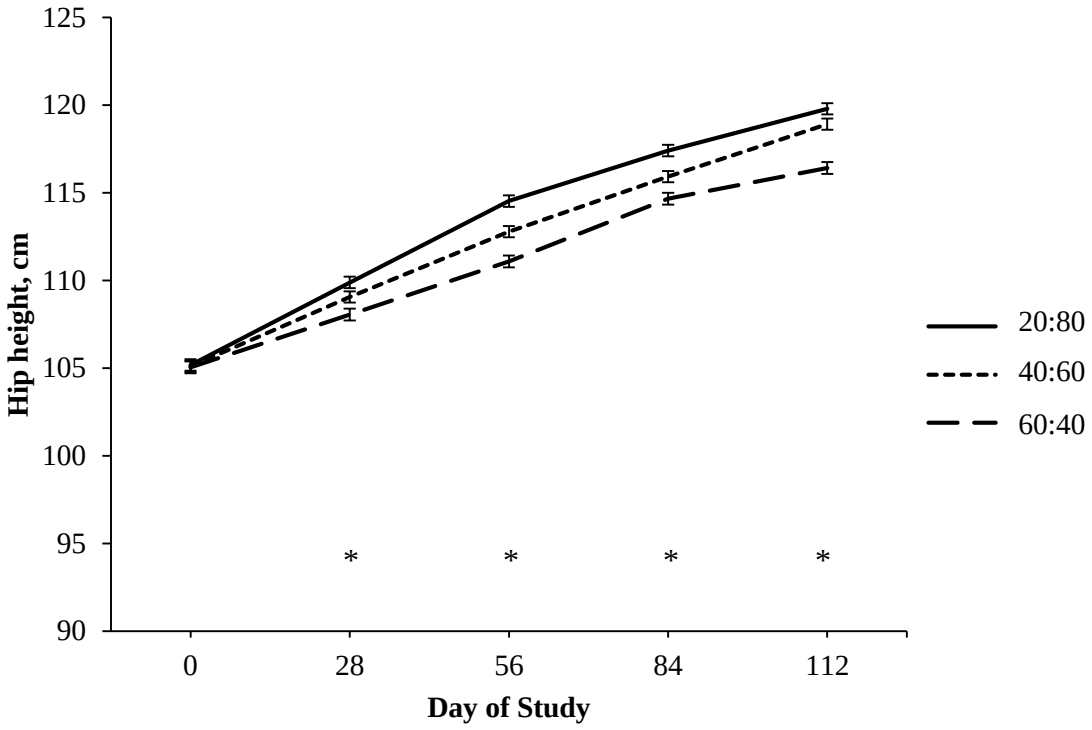

Figure 4.3. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on hip height over time. A treatment×time interaction was observed ($P < 0.01$) as heifers fed 20:80 were tallest at the hip starting at d 28 and throughout the study. 60:40 = 40% concentrate; 40:60 = 60% concentrate; 20:80 = 80% concentrate; *$P < 0.01$.

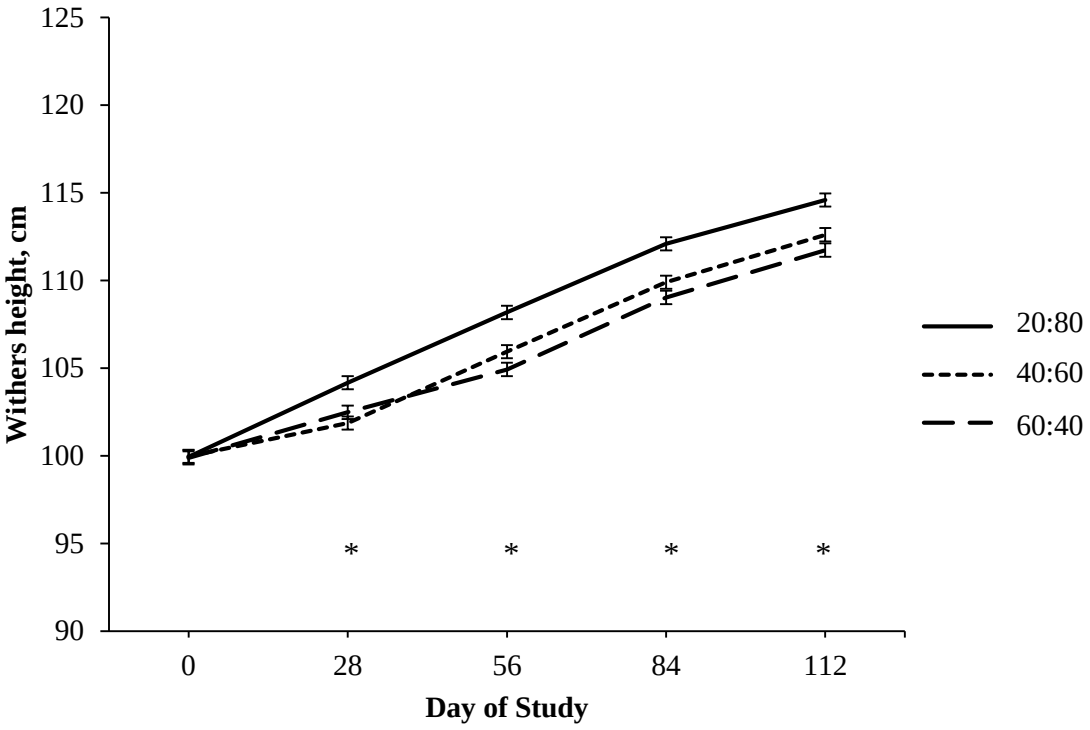

Figure 4.4. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on withers height over time. A treatment×time interaction was observed ($P < 0.01$) as heifers fed 20:80 were tallest at the withers starting at d 28 and throughout the study, whereas heifers fed 40:60 tended to be taller at the withers compared to heifers fed 60:40 starting at d 28 ($P \leq 0.10$). 60:40 = 40% concentrate; 40:60 = 60% concentrate; 20:80 = 80% concentrate; $^{*}P < 0.01$.

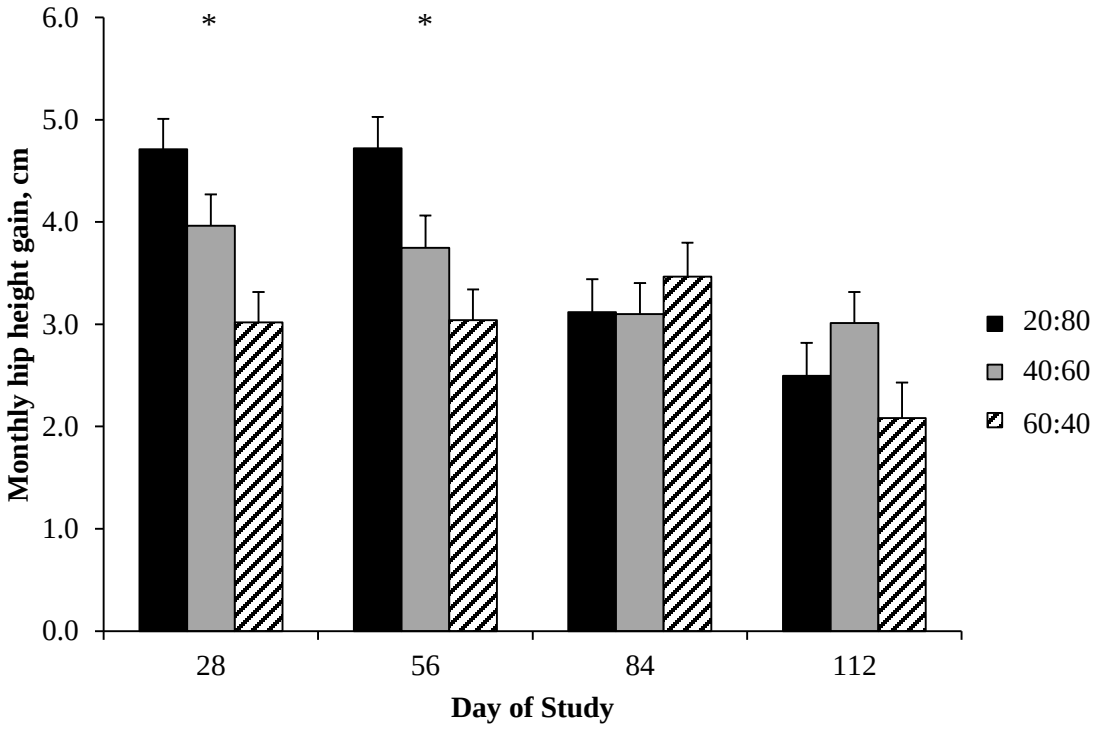

Figure 4.5. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on monthly gain of hip height over time. A treatment×time interaction was observed ($P < 0.01$) as monthly growth linearly increased as concentrate increased during the treatment period ($P < 0.01$), but growth was similar after a diet switch. 60:40 = 40% concentrate; 40:60 = 60% concentrate; 20:80 = 80% concentrate; *$P < 0.01$.

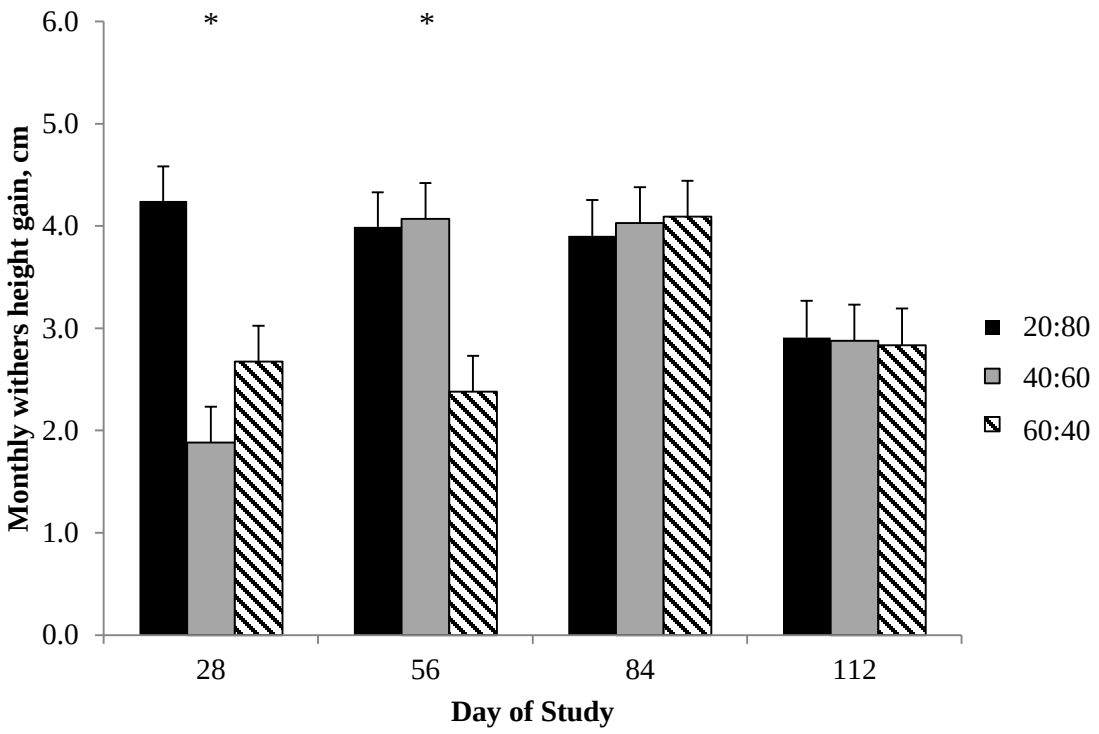

Figure 4.6. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on monthly gain of withers height over time. A treatment×time interaction was observed ($P < 0.01$) as monthly growth was greatest for heifers fed 20:80 at d 28 ($P < 0.01$) and heifers fed 20:80 and 40:60 at d 56 ($P < 0.01$), but was similar among treatments after a diet switch. 60:40 = 40% concentrate; 40:60 = 60% concentrate; 20:80 = 80% concentrate; *$P < 0.01$.

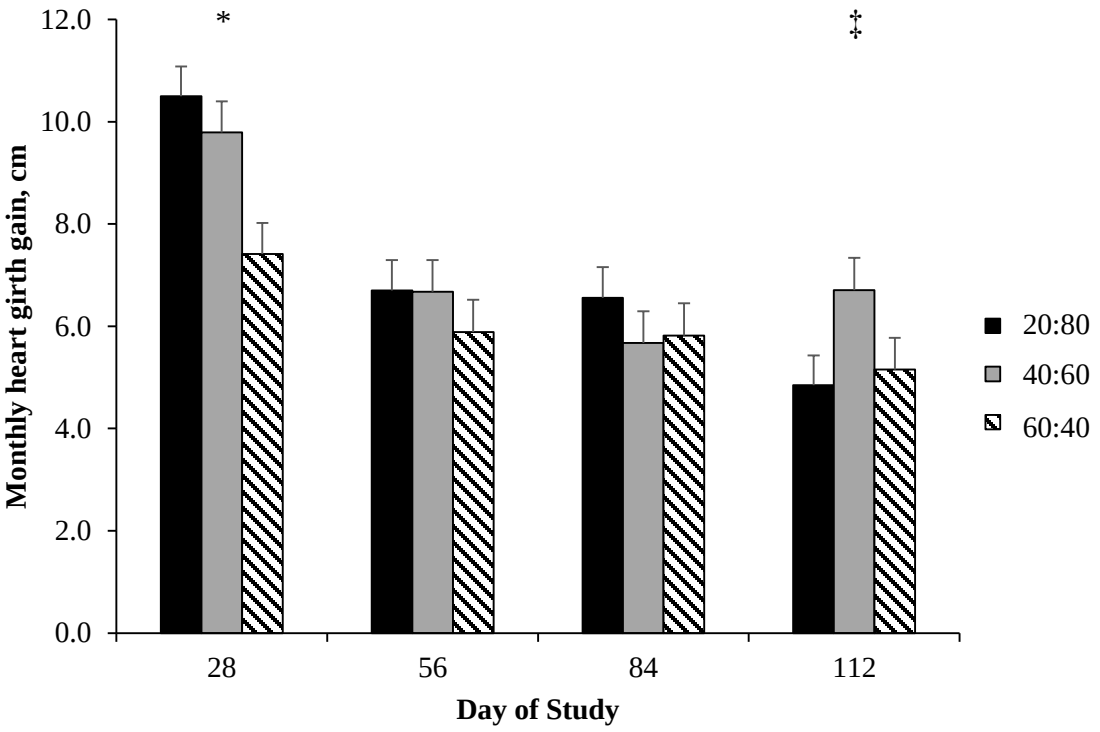

Figure 4.7. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on monthly gain of heart girth over time. A treatment×time interaction was observed ($P < 0.05$) as monthly growth was greatest for heifers fed 20:80 and 40:60 at d 28 ($P < 0.01$), but was similar among treatments after a diet switch. 60:40 = 40% concentrate; 40:60 = 60% concentrate; 20:80 = 80% concentrate; ‡$0.10 \leq P < 0.05$; *$P < 0.01$.

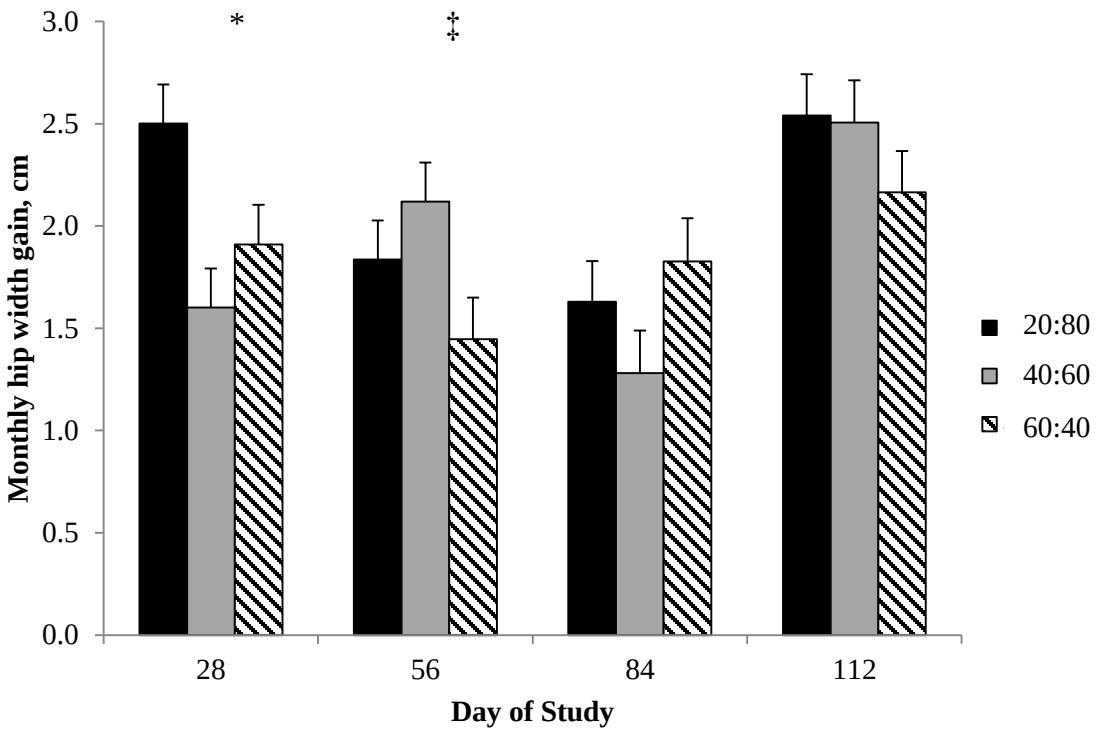

Figure 4.8. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on monthly gain of hip width over time. A treatment×time interaction was observed ($P < 0.01$) as monthly growth was greatest for heifers fed 20:80 at d 28 ($P < 0.01$) and was greatest for heifers fed 40:60 at d 56 compared to 60:40 ($P = 0.02$), but was similar among treatments after a diet switch. 60:40 = 40% concentrate; 40:60 = 60% concentrate; 20:80 = 80% concentrate; ‡$0.10 \leq P < 0.05$; *$P < 0.01$.

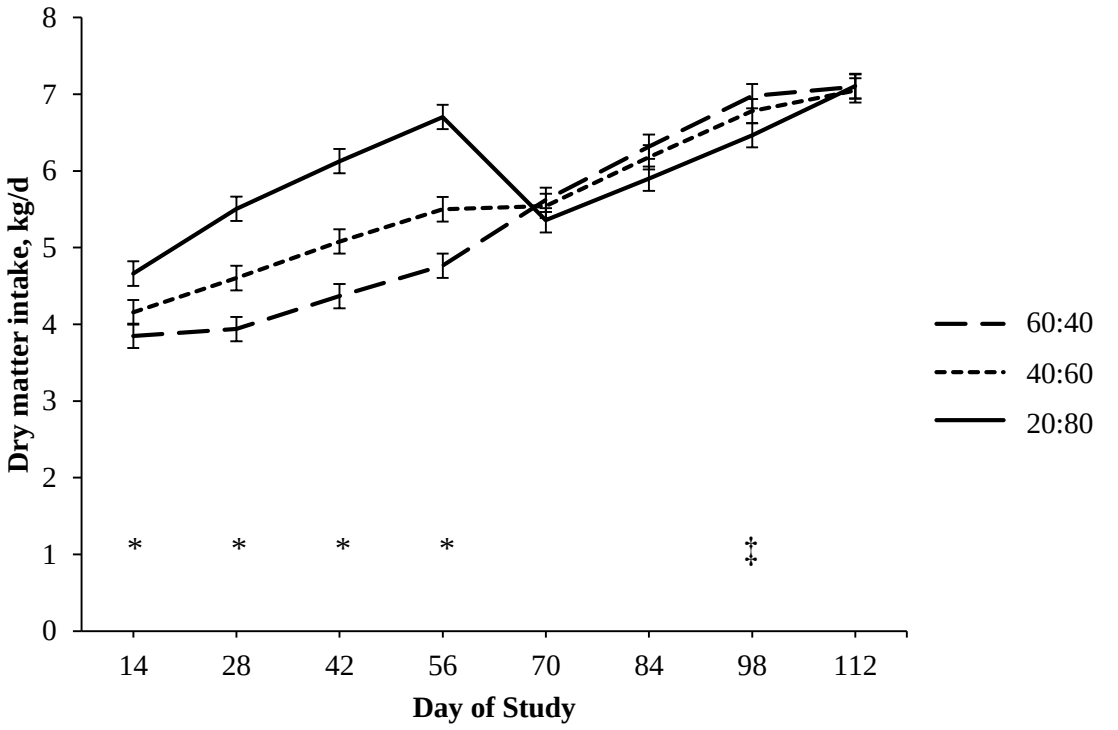

Figure 4.9. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on DM intake (kg/d) over time. A treatment×time interaction was observed ($P < 0.01$), as heifers fed 60:40 consumed the least amount of DM during the treatment period as compared to heifers fed 40:60 and 20:80; however, DM intake was similar among treatments after switching to a common diet except on d 98 when heifers fed 20:80 consumed less DM than heifers fed 60:40 ($P = 0.02$). $^{‡}0.10 \leq P < 0.05$. $^{*}P < 0.01$.

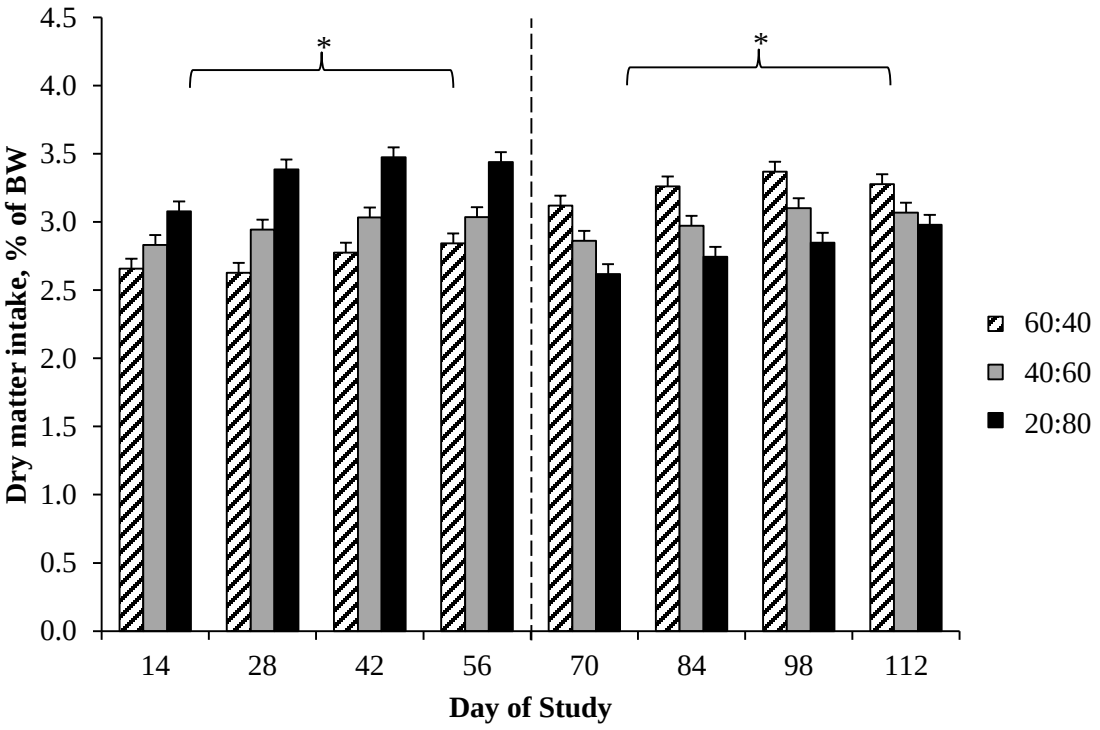

Figure 4.10. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on DM intake as a percent of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Treatment differences were not apparent overall ($P = 0.18$), however a treatment×time interaction was observed ($P < 0.01$), as heifers fed 60:40 consumed the least amount of DM during the treatment period as a percent of BW compared to heifers fed 20:80, but consumed the most DM during the grower period compared to 40:60 and 20:80. *$P <$ 0.01 at each sample day.

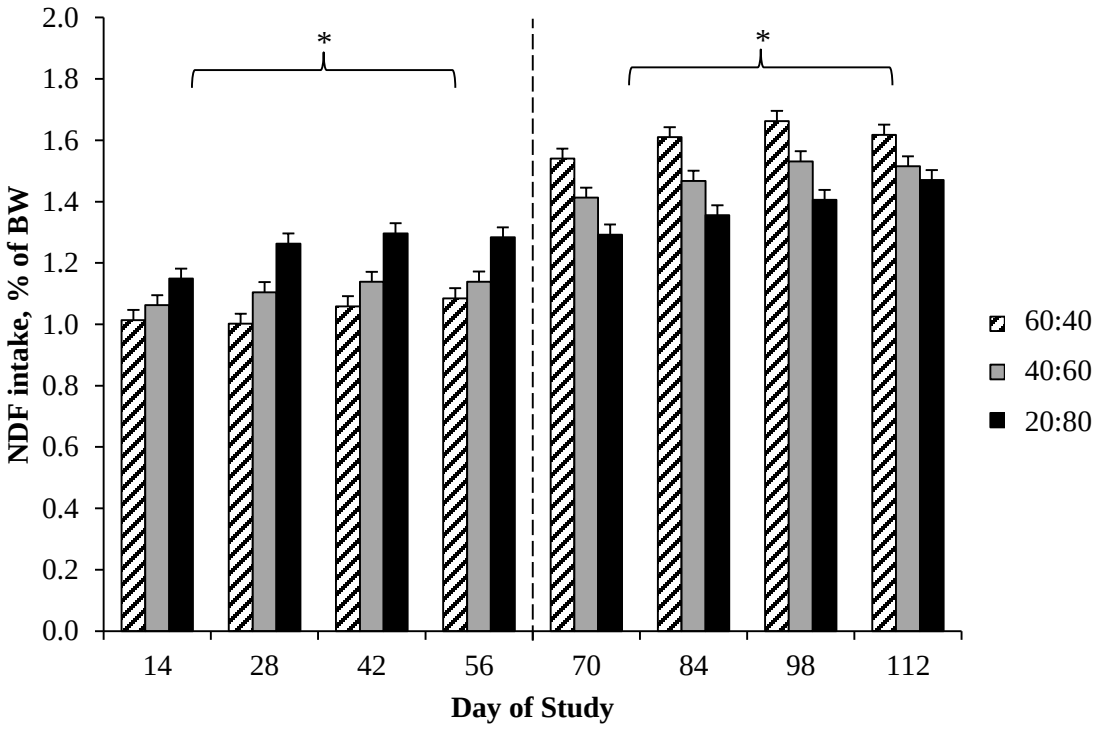

Figure 4.11. Effects of increasing concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on NDF intake (DM basis) as a percent of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Treatment differences were not apparent overall ($P = 0.46$), however a treatment×time interaction was observed ($P < 0.01$), as heifers fed 60:40 consumed the least amount of total NDF during the treatment period as a percent of BW compared to heifers fed 20:80, but consumed the most total NDF during the grower period compared to 40:60 and 20:80. *$P < 0.01$ at each sample day.

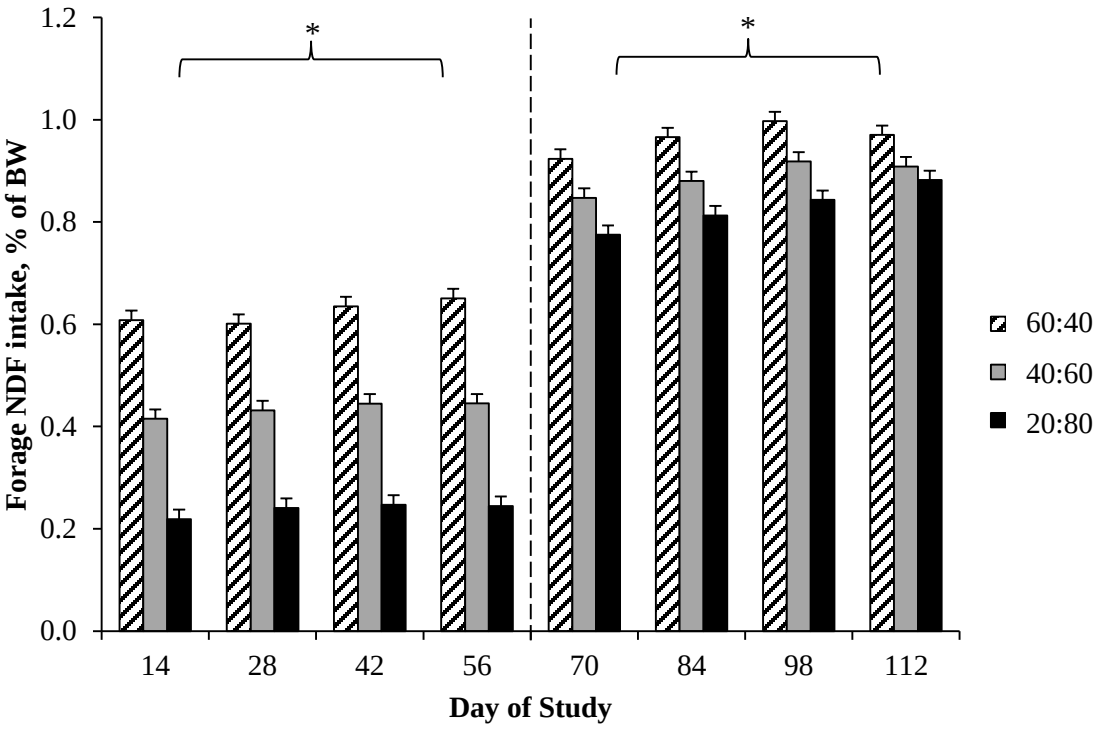

Figure 4.12. Effects of increasing level of concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on forage NDF intake (DM basis) as a percent of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Forage NDF intake increased linearly overall as grain inclusion was reduced in the treatment period ($P < 0.01$), and a treatment×time interaction was also observed overall ($P < 0.01$). As expected, forage NDF intake linearly increased as grain inclusion decreased; however, forage NDF intake was greatest throughout the grower period for heifers previously fed 60:40. *$P < 0.01$ at each sample day.

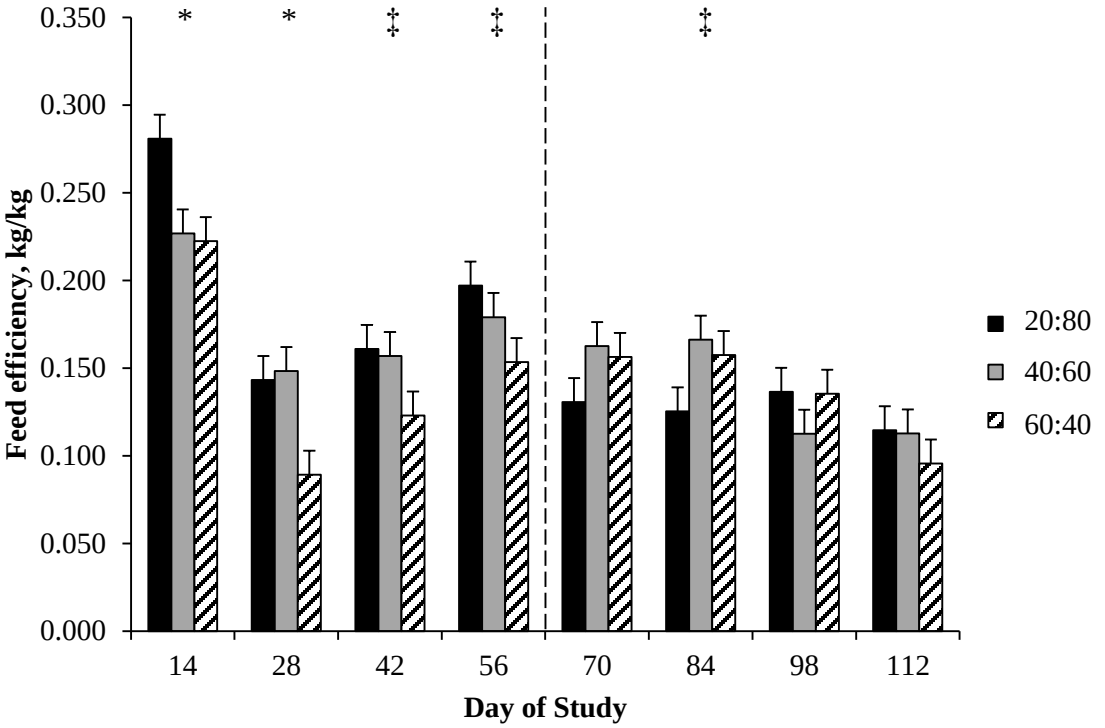

Figure 4.13. Effects of increasing level of concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on feed efficiency (G:F) over time. Vertical dashed line indicates time of diet switch relative to day of study. Feed efficiency decreased linearly overall as grain inclusion was reduced in the treatment period ($P < 0.01$), and a treatment×time interaction was also observed overall ($P < 0.01$). In general, heifers fed 20:80 exhibited greater G:F than heifers fed 60:40 during the treatment period, but G:F was greater for 60:40 following the diet switch. ‡$0.10 \leq P < 0.05$; *$P < 0.01$.

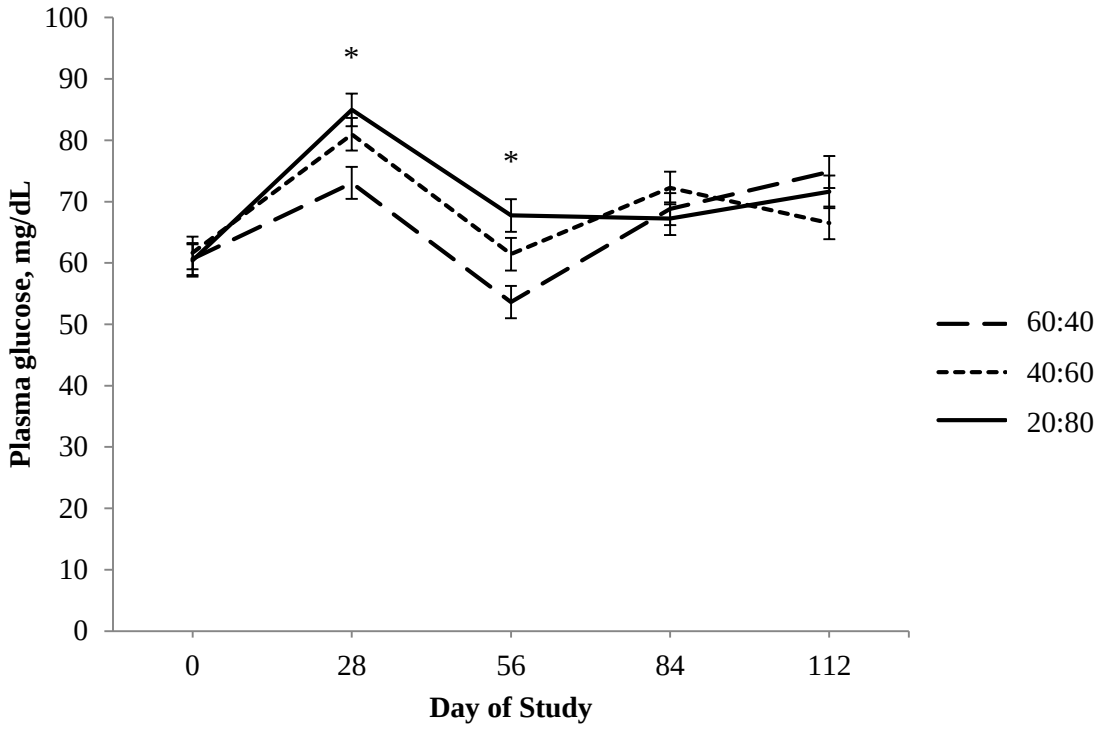

Figure 4.14. Effects of increasing level of concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on plasma glucose concentrations of growing dairy heifers. Heifers fed increasing levels of concentrate exhibited elevated glucose concentrations during the treatment period (d 0 to d 56); however, glucose did not differ between treatments following a diet switch. *$P < 0.01$.

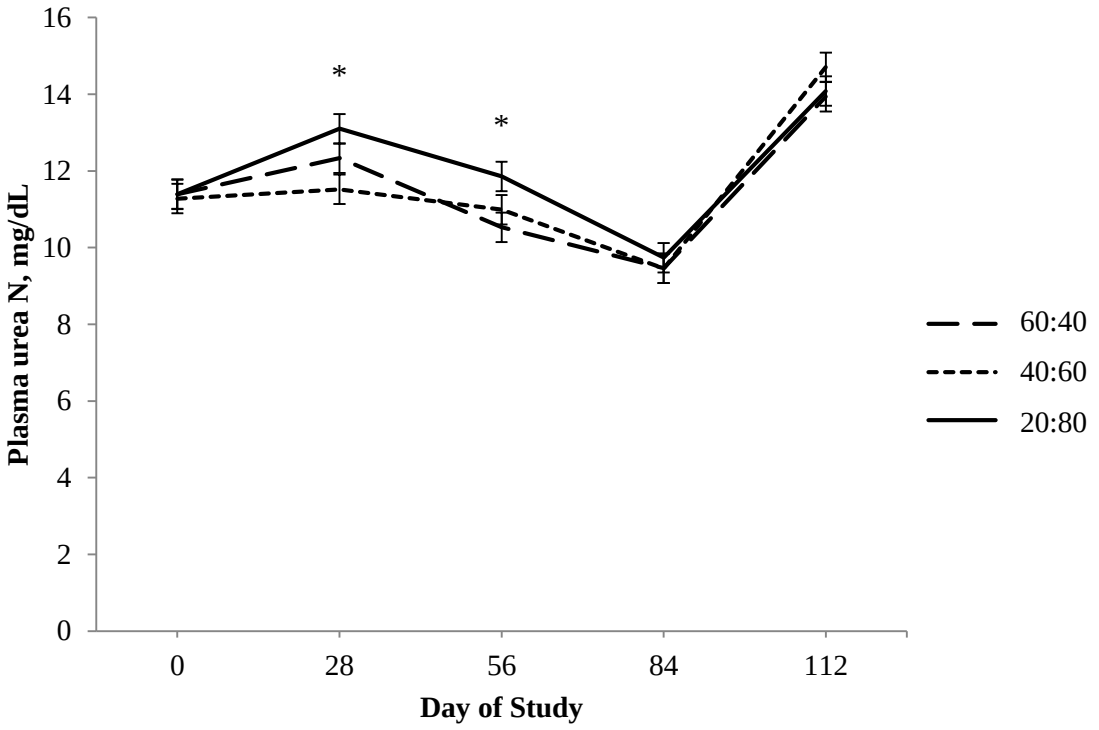

Figure 4.15. Effects of increasing level of concentrate inclusion during the treatment period (d 0 to 56) followed by a rapid switch to a common diet on plasma urea N (PUN) concentrations of growing dairy heifers. Plasma urea N increased with increasing concentrate inclusion in the diet, which was reflective of increased CP intake for heifers fed 20:80; however, PUN was similar between treatments following a switch to a common diet. *$P < 0.01$.

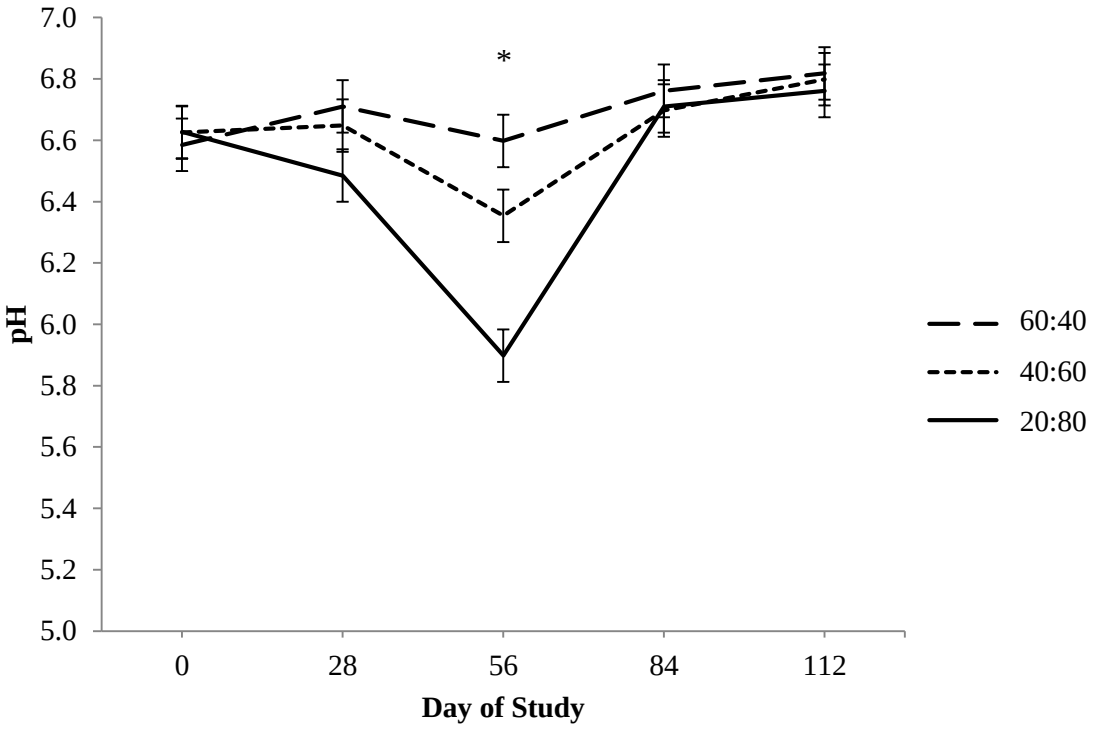

Figure 4.16. Rumen pH of heifers fed increasing levels of concentrate during the treatment period (d 0 to 56) followed by a rapid switch to a common diet. A treatment×time interaction was observed ($P < 0.01$) as pH was lowest for heifers fed 20:80 compared to 40:60 ($P < 0.01$) and 60:40 ($P < 0.01$) on d 56 of the treatment period. However, following a diet switch, rumen pH was similar among treatments on d 84 ($P = 0.86$) and d 112 ($P = 0.89$). *$P < 0.01$.

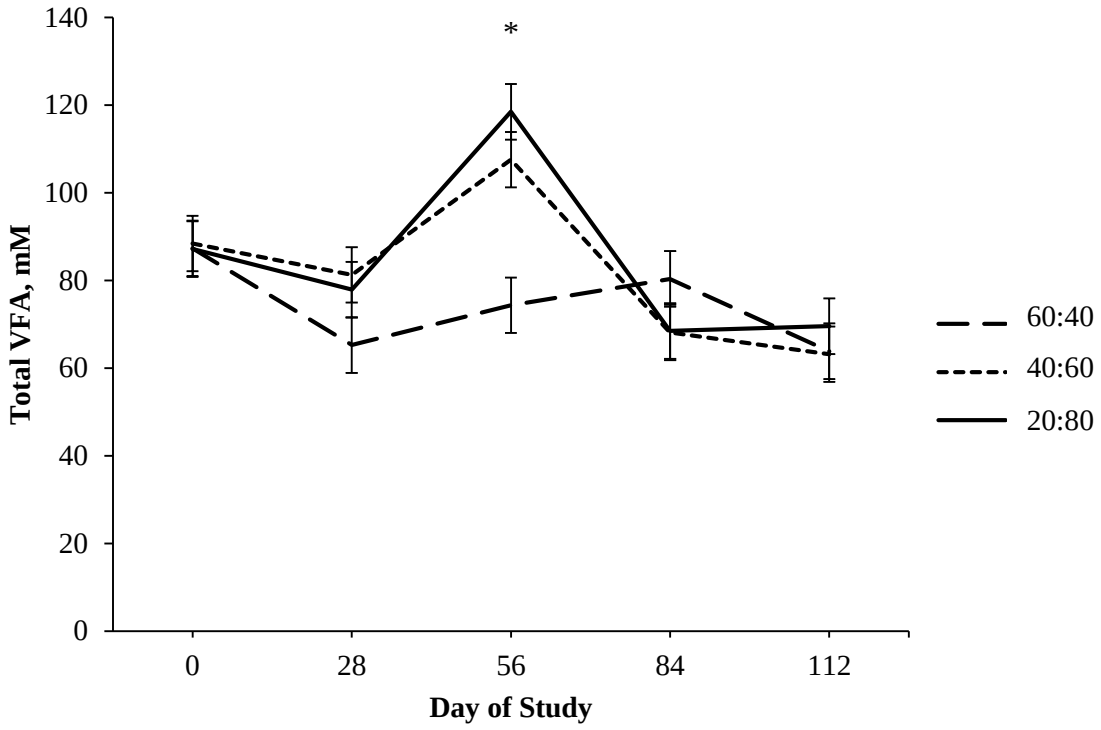

Figure 4.17. Total volatile fatty acid (VFA) concentrations for heifers fed increasing levels of concentrate during the treatment period (d 0 to 56) followed by a rapid switch to a common diet. A treatment×time interaction was observed ($P < 0.01$) as total VFA were greatest for heifers fed 20:80 and 40:60 compared to 60:40 on d 56 ($P < 0.01$), but were similar among treatments for all other sample points. Total VFA declined from d 56 to d 84 for heifers fed 20:80 ($P < 0.01$) and 40:60 ($P < 0.01$), but not 60:40 ($P = 0.51$) following a switch to a higher forage diet. *$P < 0.01$.

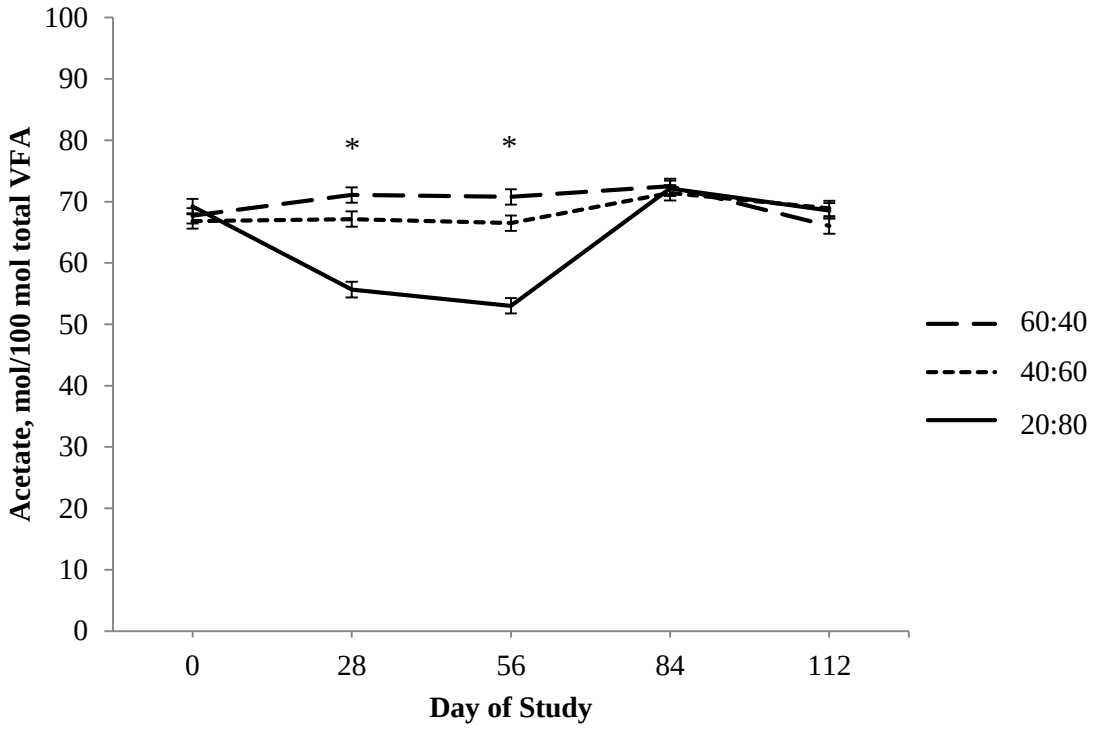

Figure 4.18. Proportion of acetate in rumen fluid for heifers fed increasing levels of concentrate during the treatment period (d 0 to 56) followed by a rapid switch to a common diet. A treatment×time interaction was observed ($P < 0.01$) as acetate was greatest for heifers fed 60:40 compared to 40:60 and 20:80 on d 28 ($P = 0.03$; $P < 0.01$) and d 56 ($P = 0.02$; $P < 0.01$) of the treatment period. Proportions of acetate increased following a switch to a higher forage diet for heifers previously fed 40:60 ($P < 0.01$) and 20:80 ($P < 0.01$), but not 60:40 ($P = 0.28$). *$P < 0.01$.

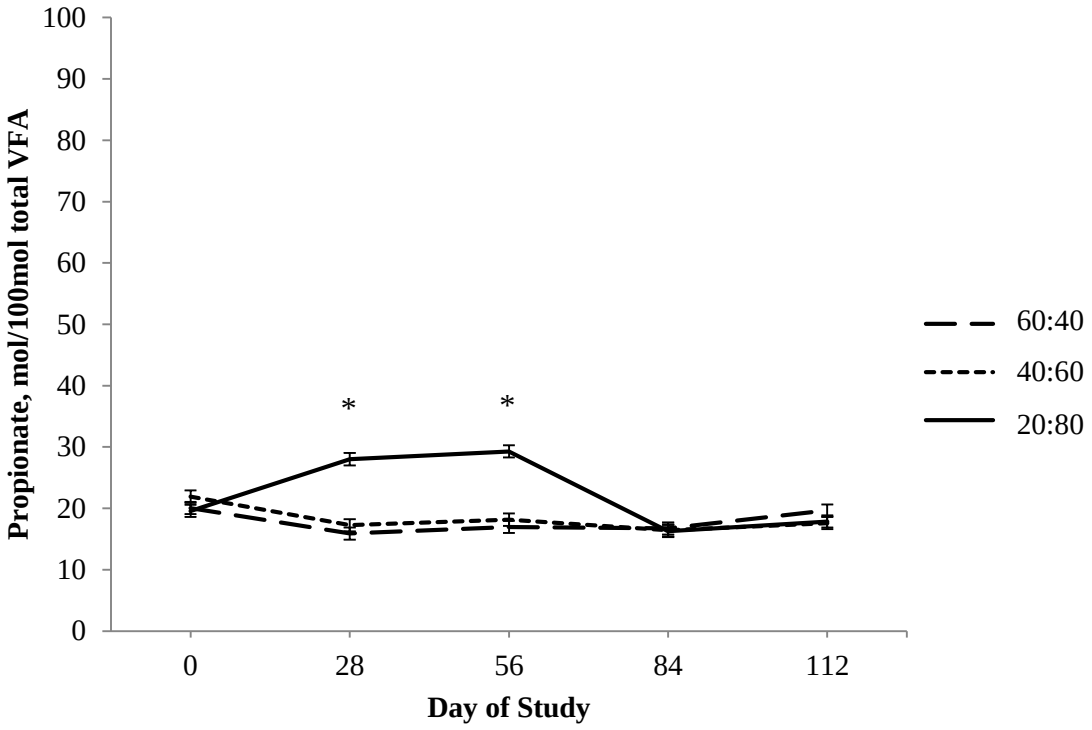

Figure 4.19. Proportion of propionate in rumen fluid for heifers fed increasing levels of concentrate during the treatment period (d 0 to 56) followed by a rapid switch to a common diet. A treatment×time interaction was observed ($P < 0.01$) as propionate was greatest for heifers fed 20:80 compared to 40:60 and 60:40 on d 28 ($P < 0.01$; $P < 0.01$) and d 56 ($P < 0.01$; $P < 0.01$) of the treatment period. Proportions of propionate decreased following a switch to a higher forage diet for heifers previously fed 20:80 ($P < 0.01$), but not 40:60 ($P = 0.13$) or 60:40 ($P = 0.83$). *$P < 0.01$.

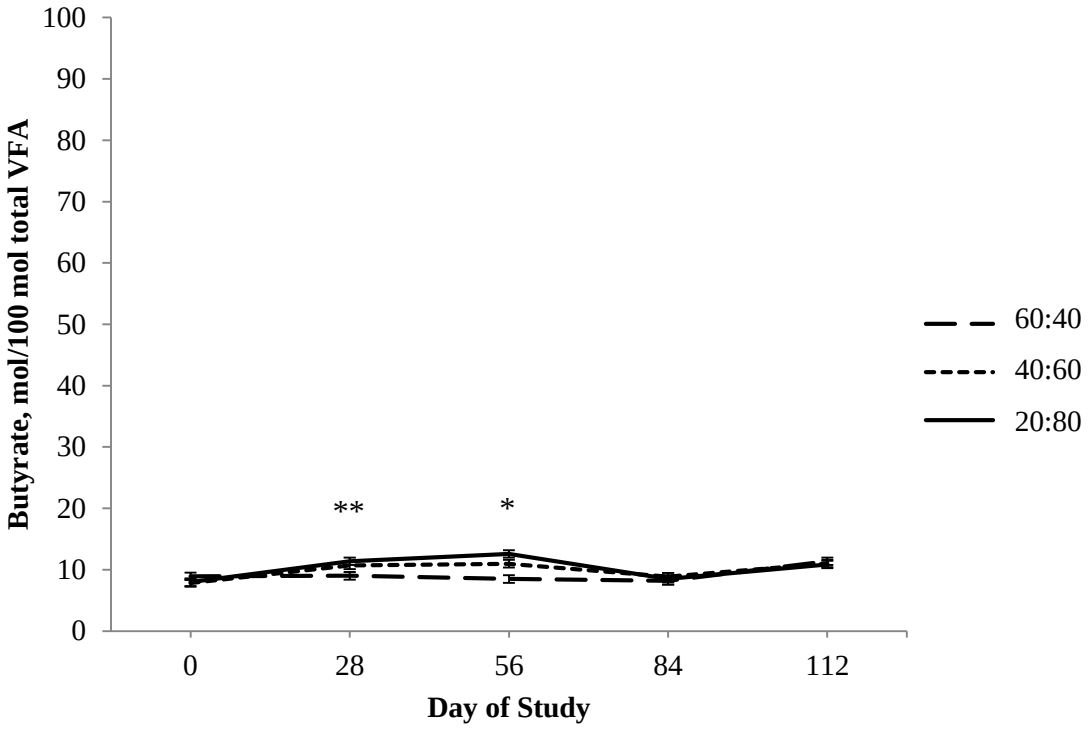

Figure 4.20. Proportion of butyrate in rumen fluid for heifers fed increasing levels of concentrate during the treatment period (d 0 to 56) followed by a rapid switch to a common diet. A treatment×time interaction was observed ($P < 0.01$) as butyrate was greatest for heifers fed 20:80 compared to 60:40 on d 28 ($P < 0.01$) and 60:40 and 40:60 on d 56 ($P < 0.01$; $P = 0.07$) of the treatment period. Proportions of butyrate decreased following a switch to a higher forage diet for heifers previously fed 20:80 ($P < 0.01$) and 40:60 ($P < 0.01$), but not 60:40 ($P = 0.69$). Additionally, butyrate increased for all treatments from d 84 to d 112 ($P < 0.01$). **$0.05 < P \leq 0.01$. *$P < 0.01$.

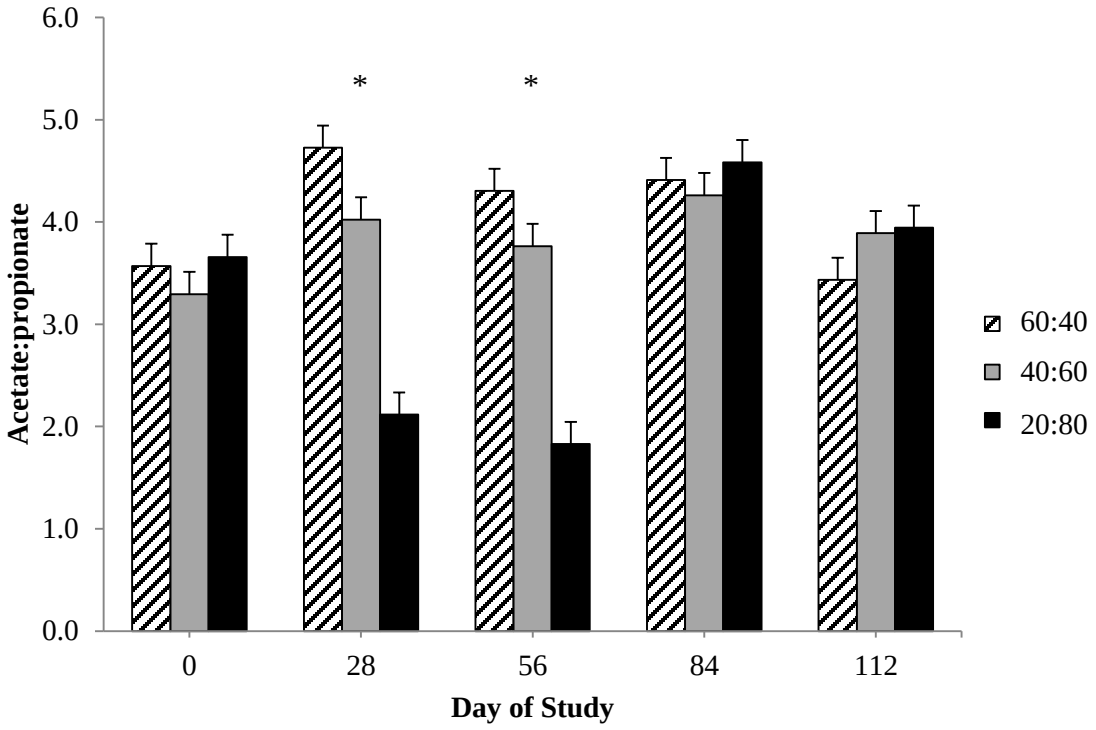

Figure 4.21. Acetate:propionate ratio in rumen fluid of heifers fed increasing levels of concentrate during the treatment period (d 0 to 56) followed by a rapid switch to a common diet. A treatment×time interaction was observed ($P < 0.01$) as acetate:propionate decreased with increasing grain inclusion on d 28 and d 56 of the treatment period, with heifers fed 60:40 exhibiting the greatest ratio compared to 40:60 ($P = 0.02$; $P = 0.08$) and 20:80 ($P < 0.01$; $P < 0.01$). Acetate:propionate increased following a switch to a higher forage diet for heifers previously fed 20:80 ($P < 0.01$) and tended to increase for 40:60 ($P = 0.06$), but not 60:40 ($P = 0.69$). *$P < 0.01$.

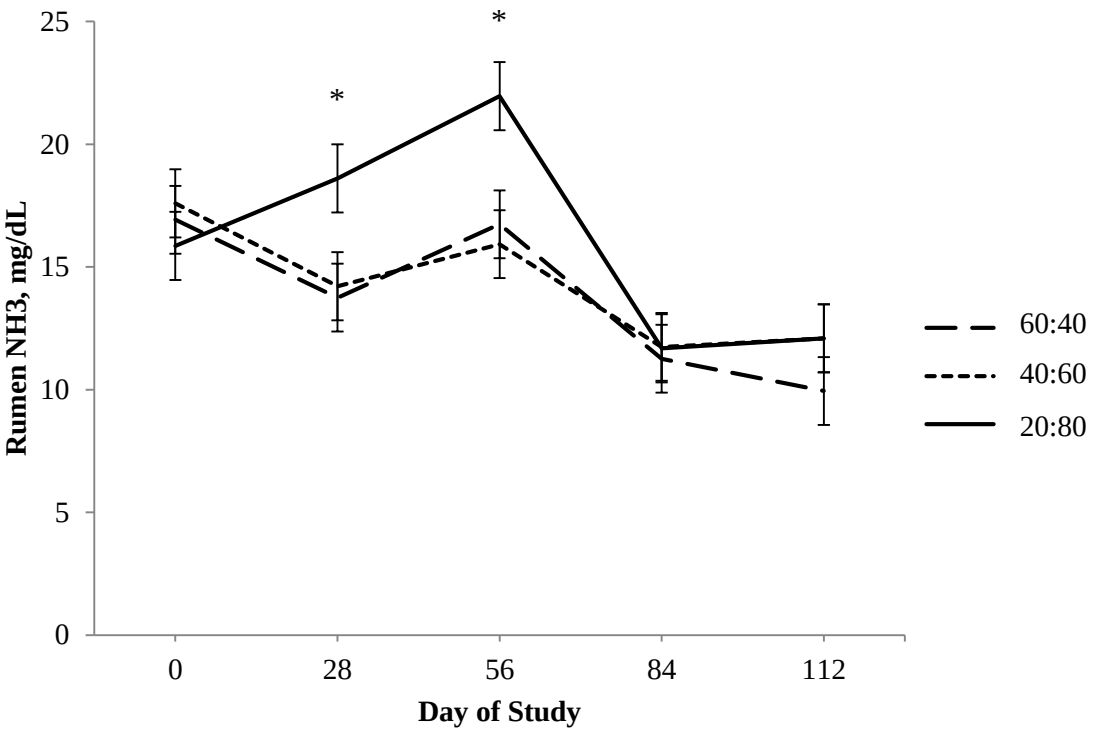

Figure 4.22. Rumen ammonia (NH3) concentrations of heifers fed increasing levels of concentrate during the treatment period (d 0 to 56) followed by a rapid switch to a common diet. A tendency for a treatment×time interaction was observed ($P = 0.06$) as NH3 increased for heifers fed 20:80 and was greatest on d 28 and d 56 compared to heifers fed 40:60 ($P = 0.03$; $P < 0.01$) and 60:40 ($P = 0.01$; $P < 0.01$). Following a diet change, rumen NH3 declined for all treatments ($P < 0.01$) and was similar among treatments on d 84 ($P = 0.96$) and d 112 ($P = 0.45$). *$P < 0.01$.

CHAPTER 5. EVALUATION OF FEED DELIVERY METHODS ON GROWTH, INTAKE, EFFICIENCY, AND RUMEN FERMENTATION CHARACTERISTICS OF PREPUBERTAL DAIRY HEIFERS

5.1 Abstract

Feeding strategies that improve growth and feed efficiency when transitioning weaned dairy heifers to the growing period may improve development to puberty. The objectives of this study were to evaluate effects of feed delivery method on growth, DMI, feed efficiency, and rumen fermentation characteristics of prepubertal dairy heifers. The study was designed with a 28 d transition and 105 d grower period. In the transition period, 90 Holstein heifers (151.0 ± 15.5 kg, 136 ± 26 d of age) were assigned to 1 of 15 pens by BW and fed a 40:60 forage:concentrate ratio (F:C) diet (DM basis) for 28 d. In the grower period, heifers were fed a 56:44 F:C diet (DM basis) for 105 d using the same feed delivery treatments. Diets were delivered using a hay feeder and grain bunk (HF), forage and grain fed side-by-side in a bunk (SBS), or a TMR. In the transition period, heifers were weighed weekly, and hip heights (HH), withers heights (WH), heart girth circumference (HGC), and body condition score (BCS) were measured every 2 wk. In the grower period, heifers were weighed every 2 wk, and HH, WH, HGC, and BCS were measured monthly. Blood and rumen fluid were collected at the beginning, middle, and end of each period. Data were analyzed by period and from d 0 to 133 as repeated measures with pen as the experimental unit. In the transition period, final BW, ADG,

and DMI were similar between feed delivery methods. Overall feed efficiency improved for HF and SBS compared to TMR (0.252 and 0.246 vs. 0.205 kg of ADG/kg of DMI, respectively). Feed delivery method did not affect HH, WH, HGC, BCS, blood metabolites, rumen NH3, or volatile fatty acids (VFA) in the transition period. At the end of the grower period, HF heifers were 15.8 and 14.0 kg heavier than SBS and TMR heifers, respectively. Average daily gains were lower for SBS and TMR compared to HF, averaging 0.83, 0.84, and 0.93 kg/d, respectively. Average DMI was greater for HF compared to SBS and TMR (7.5, 7.1, and 7.1 kg/d, respectively), resulting in similar feed efficiency between delivery methods overall. Heifers fed using HF had greater HGC than SBS and tended to have greater HGC than TMR; however, HH, WH, and BCS were not affected by feed delivery method. Blood metabolites, rumen NH3, and VFA were also not affected by feed delivery method. Results from this study showed that component feeding using a hay feeder increased ADG in both periods; however, the manner of feed delivery did not affect feed efficiency or growth in prepubertal dairy heifers during the grower period. Provision of dietary components separately in lieu of a TMR may be more appropriate earlier in the grower period for prepubertal dairy heifers to enhance performance, and switching to TMR-feeding may need to be later than 6 mo of age or for heifers greater than 260 kg of BW.

5.2 Introduction

Replacement heifers are a significant investment for dairy producers, as they are typically the second largest production cost after feed costs for the milking herd (Heinrichs et al., 2013). Up to 20% of the total cost to produce milk is attributed to

raising replacement heifers (Heinrichs, 1993), and management strategies that reduce input costs for heifers without sacrificing health or growth performance can potentially increase dairy farm profitability.

Optimizing growth of replacement heifers requires consistent supplies of digestible nutrients. It has become common practice on commercial dairy operations to deliver feed using a total mixed ration (TMR) to animals over 6 mo of age (DeVries and von Keyserlingk, 2009b). However, feed delivery methods for replacement heifers can vary between feeding dietary components separately and TMR delivery. Feed delivery using a TMR has been shown to reduce sorting behaviors against long particles in growing dairy heifers (Greter et al., 2010) and lactating cows (DeVries et al., 2007), which results in consistent intake of nutrients daily. Consistent supplies of nutrients to the rumen can optimize rumen fermentation and microbial protein synthesis (Nocek and Russell, 1988) and reduce the propensity for digestive upsets associated with drops in rumen pH due to rapid consumption of concentrates. Reductions in rumen pH below 5.5 are associated with metabolic disorders and inflammatory conditions in adult dairy cattle (Krause and Oetzel, 2006), and can have profound negative impacts on production. Provision of a TMR compared to feeding dietary components separately has been shown to increase rumination time and saliva production (Maekawa et al., 2002), thereby reducing the risk for rumen acidosis. However, when lactating Jerseys were fed concentrate separately from forage according to production level, milk production was significantly increased and feed costs were reduced compared to feeding a TMR (Gaynor et al., 1989). It is unclear if component feeding would have similar impacts on performance of growing heifers as those seen in adult dairy cattle.

Improved performance, feed efficiency, and health status of lactating cattle fed using complete TMR is prevalent in the literature (Holter et al., 1977). However, little information exists for growth and rumen fermentation characteristics of replacement dairy heifers fed using a TMR compared to component feeding. Therefore, the objective of this study was to evaluate the effects of different feed delivery methods on growth, dry matter intake (DMI), feed efficiency, and rumen fermentation characteristics of dairy heifers transitioning to higher forage diets. We hypothesized that TMR-fed dairy heifers would have improved growth performance and rumen fermentation characteristics compared to component-fed heifers when fed a similar diet during the growing period.

5.3 Materials and Methods

5.3.1 Animals and Housing

This study was conducted at the Southern Indiana Purdue Agricultural Center (SIPAC) in Dubois, IN from May 3rd to September 13th 2011using Holstein heifers sourced from Kentucky Heifers Growers, LLC of Glasgow, KY. Five d prior to initiating the study, all heifers were acclimated to facilities and a common diet consisting of a complete pelleted feed (20.5% CP; Purina Animal Nutrition LLC, Shoreview, MN) and alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) hay. All animal-related procedures were conducted in compliance with approved protocols from the Purdue Animal Care and Use Committee (PACUC no. 11-048). Ninety Holstein heifers (151.0 ± 15.5 kg, 136 ± 26 d of age) were weighed on 2 consecutive days at the beginning of the study and assigned to 1 of 15 pens (6 heifers per pen) with pens balanced by BW. Housing consisted of a naturally ventilated barn with 3.7 m x 21.9 m

pens, 3.7 m of feed bunk space, and unrestricted access to water. Pens were covered mid-way by slanted steel roofing and bedded with sawdust throughout the study as needed. Heifers were given magnet boluses and vaccinated 2 wk after beginning the experiment for bovine viral diarrhea, infectious bovine rhinotracheitis, and leptospirosis (Bovi-Shield Gold FP5 L5 HB, Pfizer Animal Health, Kalamazoo, MI) and 7 strains of *Clostridium* (Ultrabac 7, Pfizer Animal Health) and were boostered 6 wk following the first vaccination. One heifer was removed from a HF pen for rapid weight loss and subsequently died due to severe respiratory disease unrelated to feed delivery treatment.

5.3.2 Experimental Design and Treatments

The study was designed with a 28 d transition period and a 105 d grower period. Pens were randomly assigned to treatments in a completely randomized design, with heifers allocated by BW to pens. Pens were assigned to 1 of 3 feed delivery treatments: 1) feed delivered using a hay feeder (0.9 ft^3 capacity) with concentrate fed in a concrete feed bunk (HF); 2) hay and concentrate fed side-by-side in a concrete feed bunk (SBS); or 3) a total mixed ration fed in a concrete feed bunk (TMR). Transition period diets contained 40% hay and 60% concentrate on a DM basis. Hay and concentrate proportions were changed to 56% and 44%, respectively, of the diet on a DM basis for the grower period and heifers remained on previously assigned treatments until the conclusion of the study. Hay feeders used in this study were constructed to provide approximately 0.5 m of linear feed space per heifer and were placed approximately 6.1 m from the feed bunk within the pen under roofing. Feeders were also designed with a tray to catch any hay removed from the feeder but not consumed by the heifers. Ingredient

and nutrient composition of concentrate mixes and forages used in this study are presented in Table 5.1. Diets during each period were formulated according to NRC (2001) recommendations to allow 0.90 kg/d of ADG for growing Holstein heifers. Feed was initially offered at approximately 2.8% of the average pen BW and was adjusted daily to allow for *ad libitum* intake and minimize refusals (<10% daily). Hay used in the study was harvested at SIPAC in 2010 from a second cutting of an alfalfa/orchardgrass mixture. Daily concentrate allowances for HF- and SBS-fed heifers were determined by the amount of concentrate delivered to heifers fed TMR the previous day to ensure that all groups were consuming approximately the same amount of concentrate daily. Concentrate, hay for SBS, and TMR was delivered once per d at 0700 h. Hay for HF was replenished as needed to encourage *ad libitum* intake. Hay particle size was reduced using a vertical TMR mixer (Jay-Lor 4575; Jay-Lor Fabricating, Ontario, Canada) for SBS and TMR treatments. Orts from feed bunks and hay feeders were weighed and sub-sampled once per wk to determine weekly pen intakes. Feed ingredients and orts were dried at 60°C in a forced air oven, ground through a 1.0 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ), composited by month, and analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Labs, Ithaca, NY). Samples were analyzed for CP (AOAC 984.13, AOAC, 1990), NDF (Van Soest et al., 1991), ADF (AOAC 973.18, AOAC, 1990), ME (calculated from TDN in feed; NRC, 2001), and minerals (microwave digestion followed by inductively coupled plasma spectrometry; Isaac and Johnson, 1985).

5.3.3 Data Collection and Analysis

Heifers were weighed weekly during the 28 d transition period and every 2 wk during the grower period and skeletal growth measurements, including withers height (WH), hip height (HH), and heart girth circumference (HGC) were taken every 2 wk in the transition period and monthly in the grower period. Pen variances for BW (BWv) and ADG (ADGv) were calculated as described by Greter et al. (2010). Body condition scores (BCS) were assessed every 2 wk in the transition period and monthly in the grower period on a scale of 1 to 5 (1 = emaciated, 5 = obese; Edmonson et al., 1989) by 2 evaluators and averaged. Blood samples (10 mL) were collected via jugular venipuncture every 2 wk into evacuated blood tubes containing lithium heparin (BD Diagnostics, Franklin Lakes, NJ). Blood samples were kept on ice until centrifugation at 2500 x *g* for 15 min (4°C) 4 to 6 hr after collection. Plasma was aspirated following centrifugation and frozen at -20°C for later analysis. Plasma was analyzed for plasma urea N (PUN; procedure no. 0580; Stanbio Laboratory Inc., San Antonio, TX) and glucose (procedure no. 1070; Stanbio Laboratory Inc.). Rumen fluid was obtained prior to feeding as described by Dennis et al. (2012) on d 0, 14, 28 (during the transition period) 77, and 133 (during the grower period) using an esophageal tube from 2 heifers in each pen and analyzed for pH, VFA, rumen NH3, *in vitro* cellulose disappearance, and *in vitro* gas production. Rumen fluid pH was immediately determined (model HI 98130; Hanna Instruments, Ann Arbor, MI), and two 20 mL samples of fluid were acidified using 25% w/v meta-phosphoric acid (4:1 sample-to-acid ratio) and frozen at -20°C for later analysis. Rumen fluid samples were analyzed for VFA using gas chromatography on a bonded capillary column (Supelco, Bellefonte, PA; Erwin et al., 1961) and for NH3 using

the Kjeldahl procedure (FOSS Kjeltec 2300, Hoganas, Sweden; AOAC 984.13, AOAC, 1990). *In vitro* cellulose disappearance and gas production were determined using batch culture techniques described by Dennis et al. (2012). Anaerobic serum tubes (Chemglass Life Sciences, Vineland, NJ) containing 9.0 mL of basal cellulose media were inoculated with 1.0 mL of rumen fluid from each heifer, serially diluted to 10^{-8} dilution, and incubated at 37°C for 72 hr (d 0, 14, 28, and 77 only). Following incubation, total gas volume was measured and tubes were autoclaved at 125°C for 20 min to cease bacterial digestion. After autoclaving, residual cellulose was processed using the micro-NDF procedure described by Pell and Schofield (1993).

5.3.4 Statistical Analysis

Data were analyzed by period (transition or grower) and from d 0 to 133 for overall treatment effects and least-squares means are reported accordingly. Growth and intake data were analyzed as repeated measures (Littell et al., 1998) using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Treatment, time, and their interaction were included in statistical models as fixed effects and starting measurements were included in the models as covariates when statistically significant. Pen nested within treatment was considered random for growth, intake, blood metabolites, rumen pH, VFA, and NH3 models. *In vitro* cellulose disappearance and gas production were analyzed as repeated measures by heifer for each period. Means reported for cellulose disappearance and gas production were from the highest dilution with a significant difference for the response variable. Cellulose disappearance and gas production models included treatment, sample, and the interaction of the two variables as

fixed effects with heifer as a random effect. Variance-covariance matrix structures were evaluated for each model using simple, first order auto-regressive, compound symmetry, and unstructured covariance structures and were selected for each model based on the lowest Bayesian information criterion fit statistic. Least squares means and standard errors of the mean are reported on a per heifer basis and mean differences were separated using the Tukey-Kramer method. Statistical differences were considered significant at $P \leq 0.05$ and trends at $0.10 \geq P > 0.05$.

5.4 Results and Discussion

5.4.1 Heifer Weight and Growth Measurements

Growth measurements for heifers are presented in Tables 5.2 and 5.3. A significant treatment×time effect was observed overall for BW ($P < 0.01$). Heifer BW were similar among all feed delivery treatments until d 49 of the study (Figure 5.1) and thereafter, heifers fed using HF were heavier than those fed using SBS or TMR. During the transition period, BW were similar among treatments and BW on d 28 averaged 177.7 kg ($P = 0.36$). However, at the conclusion of the grower period, heifers fed using HF were, on average, 14.9 kg heavier than heifers fed using SBS or TMR ($P < 0.01$). Ending BW in the current study were 5.2 to 21.0 kg greater than those reported by Heinrichs and Losinger (1998) for heifers at a similar age, though differences in observed weights were within 1 s.d. of the mean reported in the National Dairy Heifer Evaluation Project for heifers between 8.5 and 9.5 mo of age. Weights observed in this study also agree with those of prepubertal dairy heifers fed for 1.0 kg/d ADG compared to 0.7 kg/d from 19 to 39 wk of age (Lammers et al., 1999b). Improvement in utilization of the concentrate

fraction of the diet may partially explain weight responses, as increased digestibility and improved feed efficiency for higher concentrate diets has been well-described in ruminants. Due to potential differences in hay particle length when comparing heifers fed using HF with heifers fed using SBS and TMR, passage rate may have been reduced for HF resulting in more entrapment of smaller particles in the rumen (Grant, 1997), thereby increasing ruminal digestion for HF. This may have also led to differences in total tract gut fill, which will be discussed further with respect to observed responses in DMI.

Similar to results for BW, ADG did not differ significantly between treatments in the transition period (Table 5.2). However, a treatment×time interaction was observed overall, as ADG was similar during the transition period among feed delivery treatments, but tended to be greatest for heifers fed using HF compared with SBS and TMR during the grower period ($P = 0.06$). This result likely corresponds to increased total DMI during the grower period observed for heifers fed using HF (Table 5.4). Greter et al. (2010) reported a positive correlation between ADG and social dominance for heifers fed top-dressed diets, which can potentially result in variations in performance of group-fed heifers. Similar to Greter et al. (2010), ADGv did not differ among treatments (Table 5.2), though there was a tendency ($P = 0.08$) for heifers fed using HF to have increased BWv compared with SBS and TMR, with responses initially apparent starting on d 63 of the study. Increased BWv for HF could be associated with differences in gut fill between heifers relative to weight measurements when allowed free-choice access to hay. Additionally, competition for feed resources or different feeding patterns (DeVries and von Keyserlingk, 2009b) may have contributed to increased variation; however, feeding

behavior was not evaluated in the current study. Variation in ADG was similar between feed delivery methods and differences in growth were not likely a result of competition or differences in feeding behavior.

Differences were not observed among treatments for any skeletal parameter measured (Table 5.3), with the exception of overall gain in HGC ($P = 0.01$) and BCS ($P = 0.05$) from d 0 to 133. Heifers fed using HF tended to have larger HGC compared to TMR ($P = 0.07$) and significantly larger HGC than heifers fed using SBS ($P = 0.01$) on d 133. Increased HGC for heifers fed using HF matches responses in BW, as HGC has been shown to correlate strongly with BW of growing dairy heifers (Heinrichs et al., 1992). During the transition period, BCS was similar among treatments, though all heifers significantly increased in BCS from 2.78 to 2.97 ($P < 0.01$). This response was likely due to an increase in nutrient density of the diet compared to prior nutritional plane, though a 28 d feeding period was not likely long enough to detect treatment differences. Lammers et al. (1999a) observed similar increases in BCS for heifers fed for accelerated ADG, which was likely achieved by increased ME intake compared to heifers fed for standard ADG in that study. A tendency for a treatment×time interaction was observed for BCS ($P = 0.06$), with heifers fed using HF having greater BCS compared with SBS and TMR on d 105 and greater than SBS on d 133. However, an overall reduction in BCS from the transition to the grower period was observed, likely reflective of the lower energy density and increased forage inclusion in the grower diet compared to the transition diet. Similar reductions in BCS (0.24 units of BCS) in prepubertal dairy heifers were seen by Dennis et al. (2012) after a diet change from a 40% to a 60% forage diet, although a reduction in ADG compared to the previous feeding period was also

observed in that study. Treatment responses for BCS may be related to differences in DM and ME intakes, which will be discussed below.

5.4.2 Dry Matter Intake and Feed Efficiency

Intakes of DM, CP, NDF, and ME are given in Table 5.4. During the transition period, a treatment×time interaction was observed for daily DMI (Figure 5.2), as DMI was similar on d 14, but delivering feed using HF ($P = 0.07$) and TMR ($P < 0.01$) resulted in greater DMI compared to SBS on d 28. Feed delivery method did not have an overall significant effect on DMI during the transition period ($P = 0.15$), with DMI averaging 3.9 kg/d of DMI, respectively. During the grower period, heifers fed using HF averaged 0.5 kg/d more DMI compared with SBS and TMR ($P < 0.01$). Additionally, a treatment×time interaction was observed ($P < 0.01$) as heifers fed using HF consumed more DM daily from d 63 to d 105 and d 119 to d 133 (Figure 5.2). Overall, DMI was greatest for HF compared with SBS and TMR from d 0 to 133 ($P < 0.01$). Additionally, intake of NDF, fNDF, CP, and ME on a DM basis was greatest for heifers fed HF ($P < 0.01$), corresponding to the increase in DMI. When expressed on a percent of BW basis, DMI was affected by feed delivery method during the transition period ($P = 0.03$), as heifers fed using SBS averaged 2.2% of BW as DMI compared to 2.4% of BW as DMI for heifers fed using TMR. Differences in intake as a percent of BW were most pronounced towards the end of the transition period ($P < 0.01$), averaging 2.5%, 2.6%, and 2.8% of BW as DMI for SBS, HF, and TMR, respectively, from d 14 to 28 (Figure 5.3). However, DMI as a percent of BW in the grower period was similar among feed delivery methods ($P = 0.46$). As all heifers received the same amount of concentrate

based on daily DMI of TMR-fed heifers to ensure consistent intake of nutrients from the concentrate fraction, additional DMI can be assumed to be increased hay intake for heifers fed using HF. Reasons for increased hay intake are unclear, as it is assumed that voluntary DMI would be regulated by physical fill as particle length of long-stem hay fed using HF would have been larger compared to smaller forage particles presented in a TMR and would require longer retention and chewing times to reduce particle size (Allen, 1996). As NE_g intake (Mcal/100 kg of BW) was similar overall despite a 5.6% increase in DMI for heifers fed HF compared to SBS and TMR, intake regulation for growing heifers may be more closely associated with net energy requirements than physical fill of the diet. As energy density of the diet decreased during the grower period, delivering feed using HF may have allowed heifers to better meet net energy requirements for growth by increasing voluntary hay intake in lieu of forcing hay consumption when feeding a TMR. However, this likely depends on forage digestibility and poorer quality forages may restrict intake with higher inclusion rates in the diet.

Feed efficiency, expressed as ADG/DMI (G:F), was significantly greater for HF and SBS compared to TMR during the transition period (Table 5.4). During the grower period, G:F was not influenced by feed delivery method, as DMI and ADG were increased for heifers fed using HF compared to SBS and TMR. This resulted in an overall 10.2% improvement in G:F favoring HF compared to TMR from d 0 to 133. These data suggest that, along with responses in ADG, component-fed heifers maintained intake and weight gains when transitioning to a new diet, while TMR-fed heifers reached similar ADG and efficiency towards the end of the transition period and throughout the grower period. Moya et al. (2011) reported similar G:F when comparing feeding a TMR

to component-fed diets free-choice containing barley and corn silage or barley and wheat dried distiller's grains in crossbred beef heifers, contrary to results observed during the transition period. Additionally, Greter et al. (2010) reported similar ADG and DMI between dairy heifers fed using a top-dressed diet and a TMR, suggesting similar G:F between delivery methods. However, Nocek et al. (1986) reported improved feed efficiency for post-peak lactating dairy cows receiving forage separate from grain compared to a TMR, as DMI was reduced while maintaining milk yields similar to TMR-fed cows. Differences in nutrient demands when comparing lactating cows to growing replacement heifers, as well as potential differences in feed intake regulation, may explain discrepancies in feed efficiency responses. Improvements in efficiency during the transition period for component-fed heifers may indicate an increase in retention time of diet components due to the need to reduce hay particle size. Additionally, the concentrate fraction during the transition period was entirely pelleted feed, which would have smaller particle size and faster rate of degradation in the rumen once ingested, resulting in increased nutrient utilization. In contrast, as DMI increased significantly for heifers fed using HF during the grower period and G:F was similar across treatments, it is likely that passage rate was increased enough to reduce diet digestibility in this treatment as forage inclusion and particle size of the concentrate fraction increased compared to the transition period diet. Okine and Mathison (1991) evaluated increasing intake over maintenance for dairy cows fed a 100% forage diet and the effects on passage rate and diet digestibility and observed that as intake increased to 170% of maintenance, passage rate increased and mean retention time and rumen NDF digestion tended to decrease linearly. Additionally, extent of cell wall component (NDF, ADF, hemicellulose,

cellulose) digestibility decreased with increasing intake both in the rumen and in the duodenum (Okine and Mathison, 1991). In a similar way, Zanton and Heinrichs (2008) observed a reduction in DM and NDF digestibility with increasing intake (1.25 to 2.00% of BW) for 14.5 mo old dairy heifers fed diets containing 49% NDF on a DM basis. Utilization of forages may also be limited at this age due to incomplete rumen adaptation and development; however, data evaluating rumen development beyond 84 d of age in heifers is lacking in the current literature.

Interestingly, diets were formulated according to the NRC (2001) requirements for 0.90 kg/d of ADG for Holstein heifers and estimated DMI of 5.75 kg/d at the conclusion of the study. Actual DMI observed in the current study averaged 8.63 kg/d among treatments, a 50% increase over the NRC predicted intake. The current NRC (2001) model utilizes only $BW^{0.75}$ and NE_m content of the diet when predicting intake of non-pregnant growing heifers, and does not consider other dietary or non-dietary factors. Energy equations for forages and high fiber feeds may not be accurate for growing heifers as the rumen is still developing in volume and digestive capacity after weaning. Reduced rumen volume can restrict intake and may increase passage rate in younger animals, which would reduce the net energy value of feeds at higher intakes than predicted by the model. Conversely, if net energy values of feeds are lower than predicted, more feed would be required to satisfy animal requirements, resulting in discrepancies in intake compared to predictions from the NRC (2001) model. Additionally, Hoffman et al. (2008) proposed that replacement heifers will restrict their overall intake to 1.0% of BW as NDF intake; however, in the current study, NDF intake ranged from 1.1% to 1.2% of BW during the transition period and over 2.0% of BW

during the grower period, suggesting that factors other than total dietary NDF have the potential to influence intake in replacement heifers. Forage NDF intake averaged 1.0% of BW throughout the study (Figure 5.4), which may more closely describe a dietary factor that restricts intake in growing heifers similar to what (Hoffman et al., 2008) proposed, though diets were not described in their study. While ADG was similar to NRC predicted gains in the current study, particularly for heifers fed using a TMR, the gross under-estimation of DMI by the model suggests factors other than dietary energy content are required for more accurate estimations of intake in heifers and energy equations may need to be reevaluated for growing heifers.

5.4.3 Rumen Fermentation Characteristics and Blood Metabolites

Rumen fermentation characteristics were similar among treatments overall (Table 5.5). However, treatment×time interactions were observed for individual molar proportions of VFA and rumen NH3 during each period of the study. Proportions of propionate were greatest for heifers fed HF (20.5%) on d 28 of the study compared to heifers fed SBS (18.0%) and TMR (17.7%; $P < 0.05$), but were lowest for HF (15.0%) on d 77 ($P = 0.08$). Proportions of butyrate were lowest for heifers fed HF (4.6%) on d 14 compared to heifers fed SBS (5.2%) and TMR (5.3%; $P < 0.01$). Heifers fed using SBS exhibited greater concentrations of NH3 compared to heifers fed using HF or TMR on d 14 ($P = 0.02$; Figure 5.5). This finding was unexpected given DM and CP intakes were similar among treatments during the transition period. Rumen NH3 concentrations observed during the transition period (17.9 to 23.9 mg/dL) were higher than those observed by Gabler and Heinrichs (2003b) with heifers from 153 to 196 kg of BW fed

increasing levels of CP from 16.7 to 20.1% of dietary DM. However, others have reported increased rumen degradation and substrate utilization when rumen NH3 concentrations reached 21.7 mg/dL (Mehrez et al., 1977). Therefore, elevated rumen NH3 concentrations may not be indicative of reduced N utilization in the rumen for heifers fed SBS. Following a diet change, rumen NH3 decreased from 21.6 to 14.9 mg/dL on average ($P < 0.01$), most likely due to the reduction in dietary CP. Timing of rumen fluid collection may have contributed to similar rumen pH, variable responses in other metabolite concentrations, and cellulose disappearance in batch culture, as samples were collected immediately prior to feeding. Leedle et al. (1982) evaluated diurnal variations in rumen fluid parameters and found that pH was greatest immediately prior to feeding for steers fed low-or high-forage diets (pH 6.4 and 7.1, respectively). Similarly, Li et al. (2009) observed that rumen pH was greatest and total VFA was lowest 3 h prior to feed delivery in multiparous Holstein cows. Had samples been collected up to 12 h after feeding, differences in rumen parameters may have been detected, as diurnal reductions in rumen pH (Nocek et al., 2002) due to accumulation of fermentation acids (Aschenbach et al., 2011) following feeding may have differed due to manner of feed delivery. Moya et al. (2011), however, did not detect significant differences in mean daily pH for beef heifers fed using a TMR compared to free-choice access to dietary components of the same TMR. Therefore, given a similar diet profile, feed delivery method may have little impact on fermentation profiles, though differences in diet utilization cannot be ruled out.

Plasma metabolite responses to feed delivery methods are presented in Figures 5.6 and 5.7. Glucose concentrations were affected by treatment over time ($P < 0.01$), as

glucose concentrations were elevated for heifers fed using HF and SBS compared to TMR on d 77 and d 105. Similar responses were seen for PUN as HF- and SBS-fed heifers had higher PUN on d 105 compared to TMR-fed heifers ($P < 0.01$). The observed responses in blood metabolite concentrations late in the grower period may correspond to variations in intake due to component-feeding. It is unclear, however, why the responses were only apparent 49 to 77 d following a diet change.

5.5 Summary and Conclusions

When comparing growth performance of prepubertal dairy heifers in response to different feed delivery methods, provision of the diet using a hay feeder with concentrate fed separate resulted in increased weight gains and DM intake throughout a 133 d feeding trial compared to feeding a TMR. During a 28 d transition period, feeding heifers dietary components separately, whether using a hay feeder with concentrate fed separate or hay and concentrate fed side-by-side, improved feed efficiency approximately 21.5% over feeding a TMR. Increased ADG and BCS overall for heifers fed using a hay feeder was likely a result of increased energy intake throughout the study. Rumen fermentation was not consistently influenced by feed delivery method under the conditions of the current study, but may have been a factor in differences in growth performance of component-fed heifers as propionate, butyrate, and rumen ammonia were altered early in the study. Shifts in blood metabolite patterns later in the grower period may be indicative of fluctuations in DM intake patterns often associated with component feeding, but these conclusions are speculative. From the responses observed in the current study, it appears

that feeding growing dairy heifers dietary components separately is an appropriate feed management strategy early in the grower period compared to feeding a TMR.

5.6 Acknowledgements

I would like to thank Jason Tower and the farm staff at SIPAC for daily management of the heifers on trial and support during data collection. In addition, I would like to acknowledge the support from Mike Halderman at Kentucky Heifer Growers, LLC for providing heifers used in this study. Special thanks to Dr. Jill Davidson with Purina Animal Nutrition for donation of the commercial grower pellet used in this trial and for her input on study design, analysis, and interpretation of results. I would like to thank Dr. John Patterson for help with batch culture protocols, use of equipment, and analysis for cellulose degradation and gas production data reported in this trial. Also, thanks to Marianne Bischoff-Gray for use of and technical assistance with gas chromatography and analysis of VFA raw data.

Table 5.1. Ingredient and nutrient analysis (± s.d.) of diets fed during the transition and grower phases.

Item	Transition	Grower
Ingredient, % of DM		
Alfalfa/orchardgrass hay	40	56
Pellet[1]	60	21
Cracked corn	--	15
Soybean hulls	--	8
Diet nutrient composition[2]		
DM	91.3 (1.0)	92.1 (0.2)
ME[3], Mcal/kg	2.67 (0.08)	2.38 (0.02)
NE_m[4], Mcal/kg	1.75 (0.06)	1.49 (0.01)
NE_g[5], Mcal/kg	1.02 (0.05)	0.83 (0.01)
TDN	68.0 (1.7)	62.2 (0.4)
CP	17.6 (0.9)	14.7 (0.1)
NDF	48.8 (5.8)	63.6 (1.7)
fNDF[6]	19.5	35.6
ADF	28.9 (2.2)	36.4 (1.5)
Ca	1.27 (0.00)	1.03 (0.03)
P	0.54 (0.00)	0.39 (0.02)

[1]Commercial pellet provided by Purina Animal Nutrition (Shoreview, MN) with guaranteed analysis of 18.0% CP, 1.0% crude fat, 25.0% ADF, 0.75% Ca, 0.50% P, 25.0 g/ton monensin sodium, and 5.5 g/ton diflubenzuron on an as-fed basis.
[2]All values given as a percent of DM unless otherwise stated.
[3]Calculated using the following equation: $ME = 1.01 \times [(0.04409 \times TDN) - 0.45]$.
[4]Calculated using the following equation: $NE_m = (1.37 \times ME) - (0.138 \times ME^2) + (0.0105 \times ME^3) - 1.12$.
[5]Calculated using the following equation: $NE_g = 1.42\ ME - 0.174\ ME^2 + 0.0122\ ME^3 - 1.65$.
[6]Forage NDF.

Table 5.2. Body weight and average daily gain of prepubertal dairy heifers fed common diets using different feed delivery methods.

					P-value	
Item[1]	HF	SBS	TMR	SEM	T	T×S
BW[2], kg						
d 0	151.8	149.6	151.5	2.57	0.80	--
d 28	180.7	176.1	176.4	2.57	0.36	--
d 133	275.4[a]	259.6[b]	261.4[b]	2.57	< 0.01	--
ADG[3], kg/d						
d 0 to 28	1.04	0.95	0.89	0.055	0.20	0.01
d 29 to 133	0.93[a]	0.83[b]	0.84[b]	0.029	0.06	< 0.01
BWv[4,5], kg	10.8[a,x]	8.3[ab,y]	7.5[b]	0.99	0.08	0.17
ADGv[6], kg/d	0.23	0.21	0.23	0.012	0.60	0.45

[1]HF = hay feeder; SBS = side-by-side; TMR = total mixed ration; SEM = standard error of the mean; T = treatment; T×S = interaction of treatment by sample.
[2]Body weight.
[3]Average daily gain.
[4]Variance in BW; BWv was calculated by averaging the absolute difference between individual heifer BW and pen mean BW.
[5]Initial measurement included in the model as a covariate.
[6]Variance in ADG; ADGv calculated by averaging the absolute difference between individual heifer ADG and pen mean ADG.
[ab]Means differ at $P \leq 0.05$ level.
[xy]Means tend to differ at $0.10 \leq P < 0.05$ level.

Table 5.3. Skeletal measurements of prepubertal dairy heifers fed common diets using different feed delivery methods.

Item[1]	HF	SBS	TMR	SEM	*P*-value
Hip height, cm					
d 0	107.0	106.4	106.7	0.65	0.80
d 28	111.0	110.7	111.2	0.67	0.89
d 133	121.0	121.5	121.6	0.64	0.81
Overall gain	14.4	14.9	14.9	0.38	0.55
Withers height, cm					
d 0	101.0	101.1	100.9	0.63	0.98
d 28	105.7	105.6	105.9	0.66	0.93
d 133	116.1	116.4	116.3	0.62	0.94
Overall gain	15.1	15.3	15.4	0.40	0.85
Heart girth, cm					
d 0	123.0	123.3	123.4	0.72	0.92
d 28	131.2	130.3	130.4	0.74	0.66
d 133	$149.5^{a,x}$	146.9^{b}	$147.6^{b,y}$	0.71	0.03
Overall gain	26.4^{a}	23.7^{b}	24.2^{b}	0.59	0.01
BCS[2,3], 1 to 5 scale					
d 0	2.80	2.75	2.78	0.030	0.47
d 28	2.99	2.97	2.94	0.030	0.46
d 133	2.71^{a}	$2.59^{b,y}$	$2.66^{a,x}$	0.030	0.02
Overall change	-0.17^{b}	-0.29^{a}	-0.22^{ab}	0.031	0.05

[1]HF = hay feeder; SBS = side-by-side; TMR = total mixed ration; SEM = standard error of the mean.
[2]Body condition score.
[3]Inital measurement included in the model as a covariate.
[abc]Means differ at $P \leq 0.05$ level.
[xyz]Means tend to differ at $0.10 \leq P < 0.05$ level.

Table 5.4. Intake and feed efficiency of prepubertal dairy heifers fed common diets using different feed delivery methods.

					P-value	
Item[1]	HF	SBS	TMR	SEM	T	T×S
DMI[2]						
Overall						
kg/d	6.74[a]	6.36[b]	6.40[b]	0.073	< 0.01	< 0.01
% of BW[3]	3.03	2.98	2.99	0.032	0.57	< 0.01
d 0 to 28						
kg/d	3.97	3.76	4.03	0.093	0.15	0.01
% of BW	2.30[ab]	2.23[b]	2.38[a]	0.035	0.03	< 0.01
d 29 to 133						
kg/d	7.53[a]	7.11[b]	7.08[b]	0.088	< 0.01	< 0.01
% of BW	3.23	3.20	3.16	0.042	0.46	0.26
NDF intake						
Overall						
kg/d	4.16[a]	3.93[b]	3.94[b]	0.045	< 0.01	< 0.01
% of BW	1.85	1.83	1.82	0.022	0.56	0.03
d 0 to 28						
kg/d	1.93	1.83	1.96	0.045	0.15	0.01
% of BW	1.12[ab]	1.09[b]	1.16[a]	0.017	0.03	< 0.01
d 29 to 133						
kg/d	4.79[a]	4.52[b]	4.51[b]	0.056	< 0.01	< 0.01
% of BW	2.06	2.04	2.01	0.027	0.46	0.26
fNDF intake[4]						
Overall						
kg/d	2.26[a]	2.13[b]	2.14[b]	0.025	< 0.01	< 0.01
% of BW	1.00	0.98	0.98	0.011	0.53	0.07
d 0 to 28						
kg/d	0.77	0.73	0.79	0.018	0.15	0.01
% of BW	0.45[ab]	0.44[b]	0.47[a]	0.007	0.03	< 0.01
d 29 to 133						
kg/d	2.68[a]	2.53[b]	2.52[b]	0.031	< 0.01	< 0.01
% of BW	1.15	1.13	1.14	0.015	0.46	0.26
ME intake						
Overall						
Mcal/d	16.3[a]	15.4[b]	15.5[b]	0.18	< 0.01	< 0.01
Mcal/100 kg of BW	7.4	7.3	7.3	0.08	0.58	< 0.01
d 0 to 28						
Mcal/d	10.6	10.1	10.8	0.25	0.15	0.01
Mcal/100 kg of BW	6.2[ab]	6.0[b]	6.4[a]	0.09	0.03	< 0.01
d 29 to 133						
Mcal/d	17.9[a]	16.9[b]	16.8[b]	0.21	< 0.01	< 0.01

Table 5.4. continued

Mcal/100 kg of BW	7.7	7.6	7.5	0.10	0.46	0.26
NE_g intake						
Overall						
Mcal/d	5.8[a]	5.5[b]	5.5[b]	0.06	< 0.01	< 0.01
Mcal/100 kg of BW	2.6	2.6	2.6	0.03	0.58	< 0.01
d 0 to 28						
Mcal/d	4.0	3.8	4.1	0.09	0.15	0.01
Mcal/100 kg of BW	2.3[ab]	2.3[b]	2.4[a]	0.04	0.03	< 0.01
d 29 to 133						
Mcal/d	6.3[a]	5.9[b]	5.9[b]	0.07	< 0.01	< 0.01
Mcal/100 kg of BW	2.7	2.7	2.6	0.04	0.46	0.26
CP intake						
Overall						
kg/d	1.02[a]	0.96[b]	0.97[b]	0.011	< 0.01	< 0.01
% of BW	0.46	0.45	0.46	0.005	0.58	< 0.01
d 0 to 28						
kg/d	0.70	0.66	0.71	0.016	0.15	0.01
% of BW	0.41[ab]	0.39[b]	0.42[a]	0.006	0.03	< 0.01
d 29 to 133						
kg/d	1.11[a]	1.05[b]	1.04[b]	0.013	< 0.01	< 0.01
% of BW	0.48	0.47	0.47	0.006	0.46	0.26
Feed efficiency[5]						
Overall	0.151[a]	0.145[ab,x]	0.137[b,y]	0.003	0.03	< 0.01
d 0 to 28	0.252[a]	0.246[a]	0.205[b]	0.014	0.06	0.33
d 29 to 133	0.123	0.116	0.117	0.003	0.41	< 0.01

[1]HF = hay feeder; SBS = side-by-side; TMR = total mixed ration; SEM = standard error of the mean; T = treatment; T×S = interaction of treatment by sample.
[2]Dry matter intake.
[3]Body weight.
[4]Forage NDF intake.
[5]Feed efficiency expressed as kg of ADG per kg of daily DMI.
[ab]Means differ at $P \leq 0.05$ level.
[xy]Means tend to differ at $0.10 \leq P < 0.05$ level.

Table 5.5 Rumen fermentation characteristics of prepubertal dairy heifers fed common diets using different feed delivery methods.

					P-value	
Item	HF	SBS	TMR	SEM	T	T×S
Rumen pH						
d 0 to 28	6.65	6.65	6.64	0.042	0.98	0.54
d 29 to 133	6.95	6.91	6.90	0.051	0.77	0.15
Rumen NH3, mg/dL						
d 0 to 28	16.4	19.4	17.3	1.43	0.36	0.08
d 29 to 133	19.5	19.3	18.4	0.96	0.71	0.04
Total VFA[2], mM						
d 0 to 28	58.0	60.8	60.2	4.26	0.88	0.27
d 29 to 133	58.6	59.0	59.4	4.36	0.99	0.05
Molar proportion of VFA[3]						
d 0 to 28						
Acetate	72.9	73.8	73.0	0.80	0.65	0.07
Propionate	17.9	16.6	17.0	0.79	0.49	0.09
Butyrate	5.0	5.2	5.5	0.14	0.11	0.01
A:P[4]	4.40	4.60	4.49	0.254	0.85	0.57
d 29 to 133[5]						
Acetate	73.0	73.1	73.3	0.40	0.86	0.01
Propionate	16.5	16.7	16.3	0.40	0.82	< 0.01
Butyrate	5.8	5.7	6.0	0.15	0.31	0.04
A:P	4.57	4.50	4.62	0.117	0.77	< 0.01
Gas production[6]						
d 0 to 28[5]	1.2	1.5	1.2	0.15	0.34	0.28
d 29 to 77	3.2	3.6	3.6	0.18	0.22	0.20
Cellulose disappearance[7]						
d 0 to 28[5]	3.6	3.1	2.8	1.11	0.87	0.40
d 29 to 77	4.4	3.8	3.9	1.21	0.93	0.22

[1]HF = hay feeder; SBS = side-by-side; TMR = total mixed ration; SEM = standard error of the mean; T = treatment; T×S = interaction of treatment by sample.
[2]Volatile fatty acids; Individual VFA measured include acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate.
[3]Molar proportion expressed as mol individual VFA/100 mol total VFA.
[4]Acetate:propionate ratio.
[5]Inital measurements included in the model as a covariate.
[6]Reported as mL of total headspace gas from batch culture dilutions of 10^{-8}.
[7]Reported as percent disappearance of cellulose from batch culture dilutions of 10^{-8}.

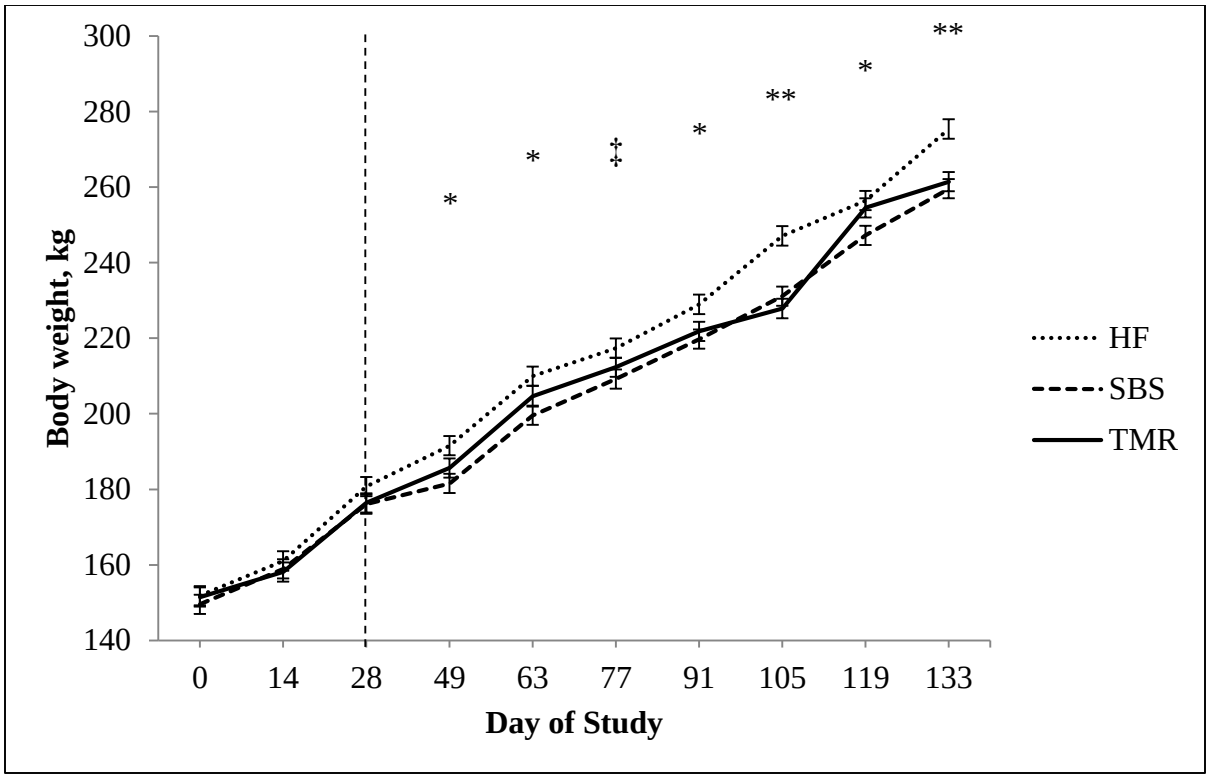

Figure 5.1 Body weight of prepubertal dairy heifers fed identical diets using a hay feeder (HF), side-by-side in a feedbunk (SBS), or a total mixed ration (TMR). Vertical dashed line indicates a switch to a 56:44 forage-to-concentrate diet (from a 40:60) with same feed delivery treatments. A treatment×time interaction was observed ($P < 0.01$). $^{‡}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$.

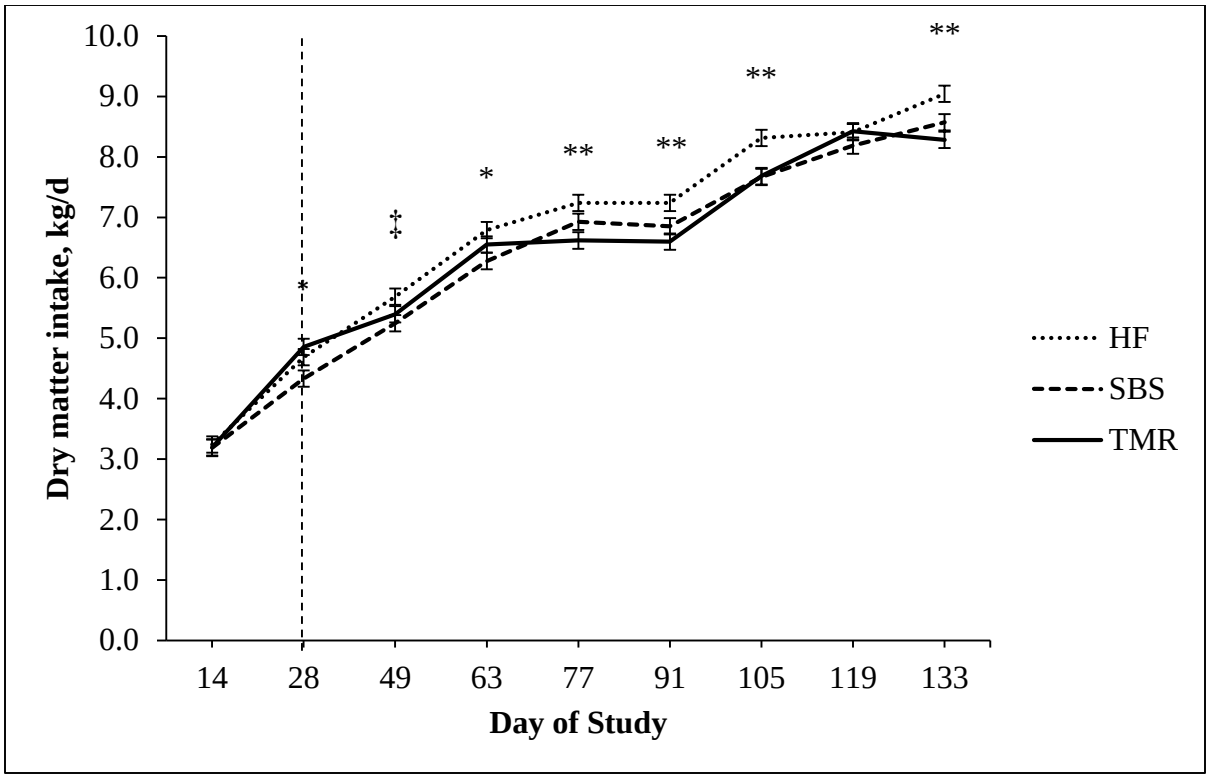

Figure 5.2 Dry matter intake (kg/d) for heifers fed identical diets using a hay feeder (HF), side-by-side in a feedbunk (SBS), or a total mixed ration (TMR). Vertical dashed line indicates a switch to a 56:44 forage-to-concentrate diet (from a 40:60) with same feed delivery treatments. A treatment×time interaction was observed ($P < 0.01$). $^{‡}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$.

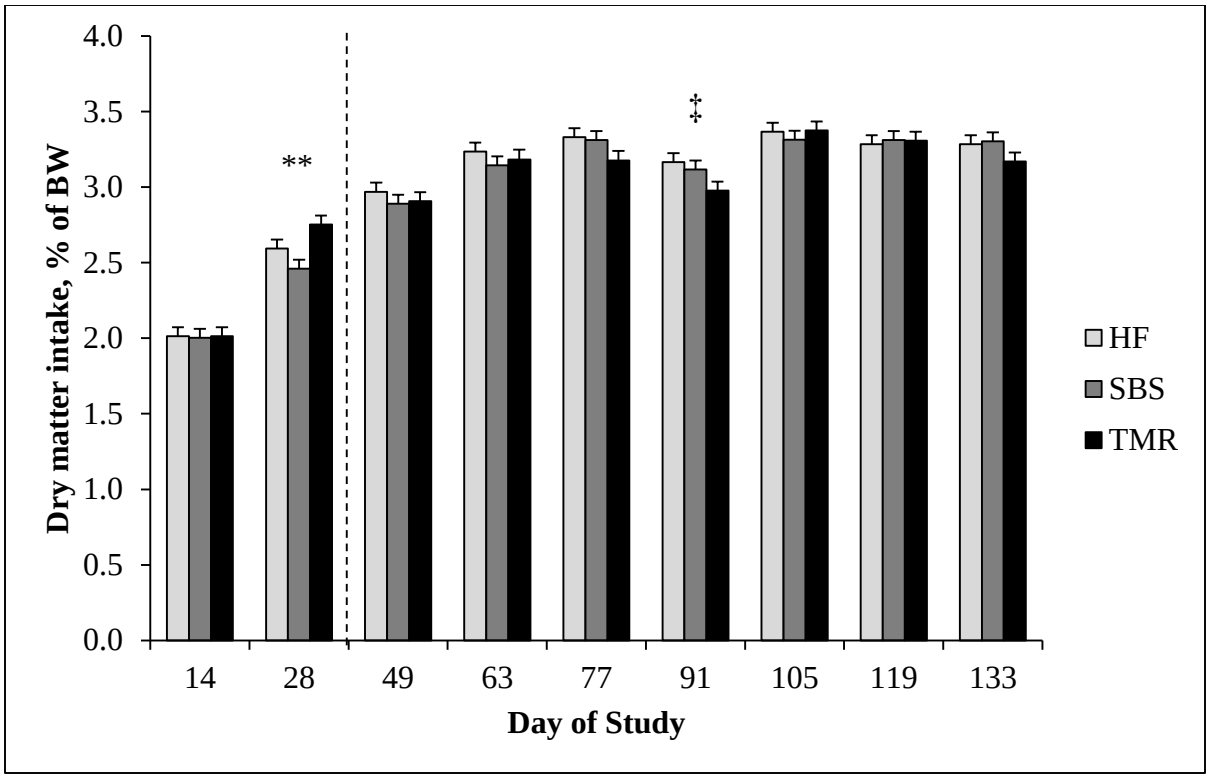

Figure 5.3 Dry matter intake (% of BW) for heifers fed identical diets using a hay feeder (HF), side-by-side in a feedbunk (SBS), or a total mixed ration (TMR). Vertical dashed line indicates a switch to a 56:44 forage-to-concentrate diet (from a 40:60) with same feed delivery treatments. A treatment×time interaction was observed ($P < 0.01$). $^{‡}P < 0.10$; $^{**}P < 0.01$.

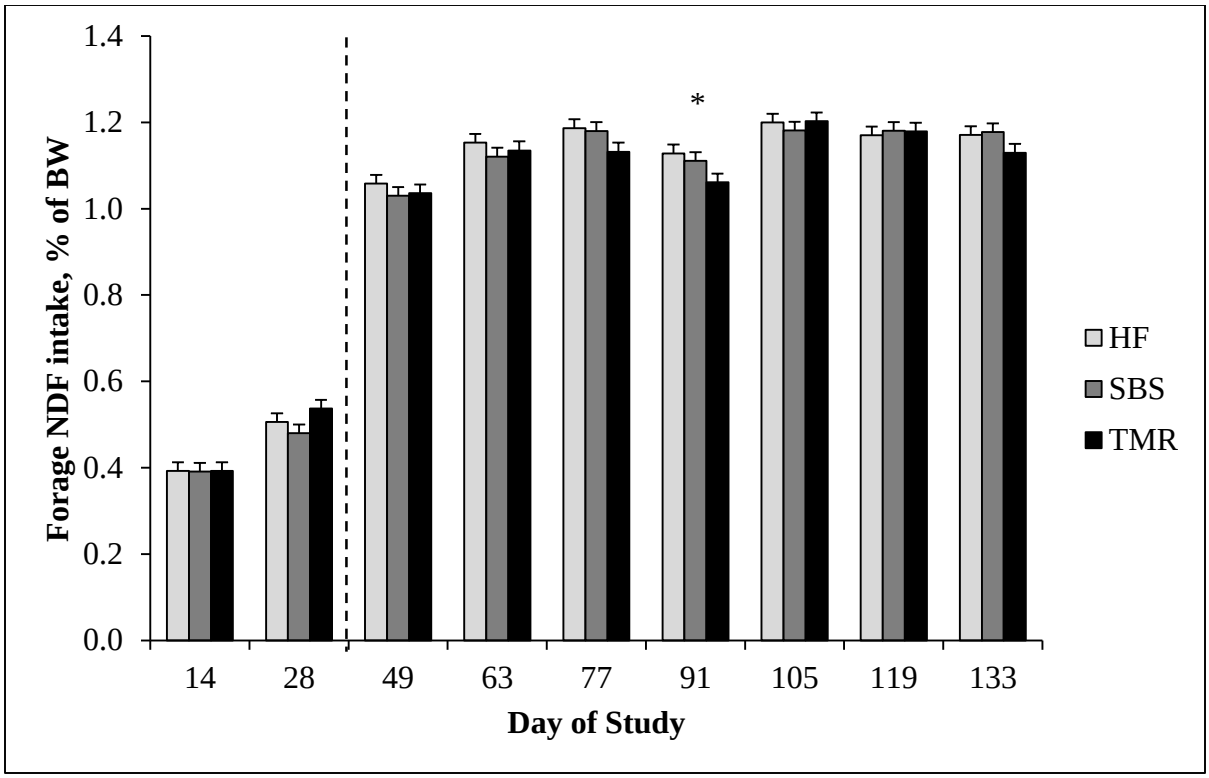

Figure 5.4 Forage NDF intake (% of BW) for heifers fed identical diets using a hay feeder (HF), side-by-side in a feedbunk (SBS), or a total mixed ration (TMR). Vertical dashed line indicates a switch to a 56:44 forage-to-concentrate diet (from a 40:60) with same feed delivery treatments. A tendency for a treatment×time interaction was observed ($P = 0.07$). $^{*}P < 0.05$.

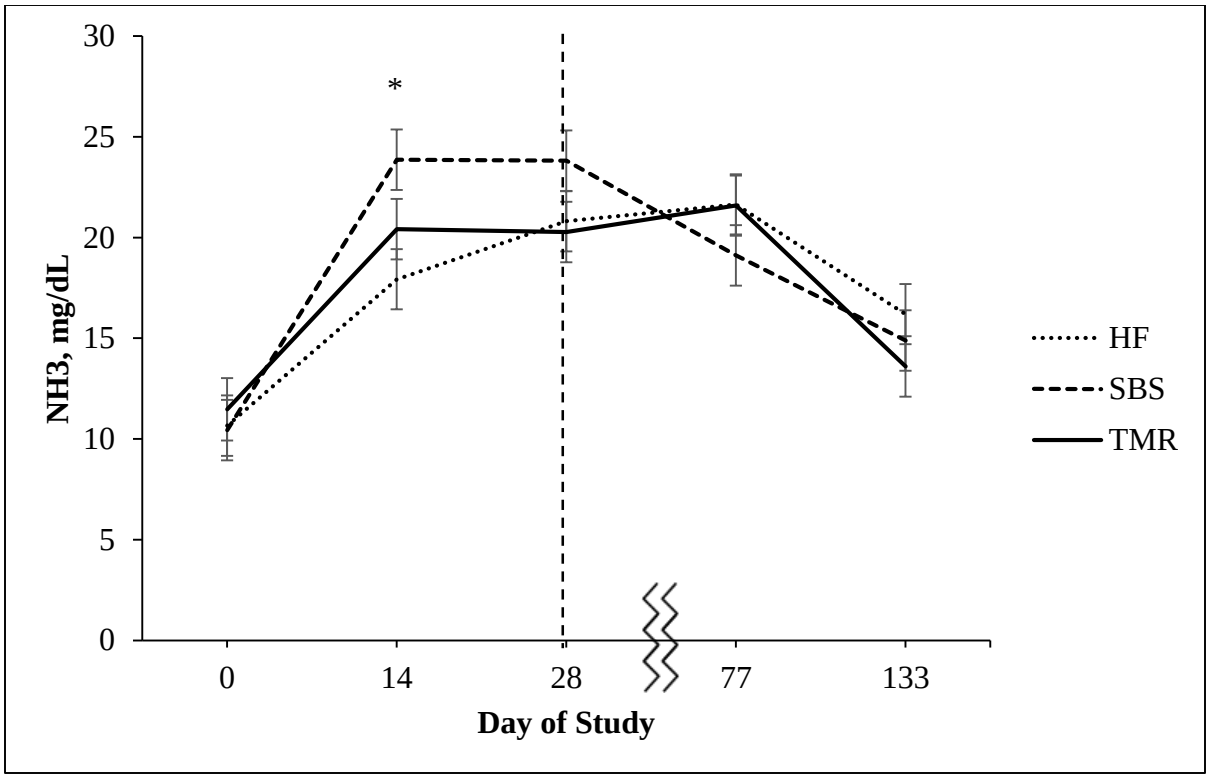

Figure 5.5 Rumen ammonia (NH3) concentrations for heifers fed diets delivered using a hay feeder and feed bunk (HF), side-by-side in a feed bunk (SBS), or a total mixed ration (TMR). Vertical dashed line indicates a switch to a 56:44 forage-to-concentrate diet (from a 40:60) with same delivery treatments. A treatment×time interaction was observed ($P < 0.01$). *$P < 0.05$.

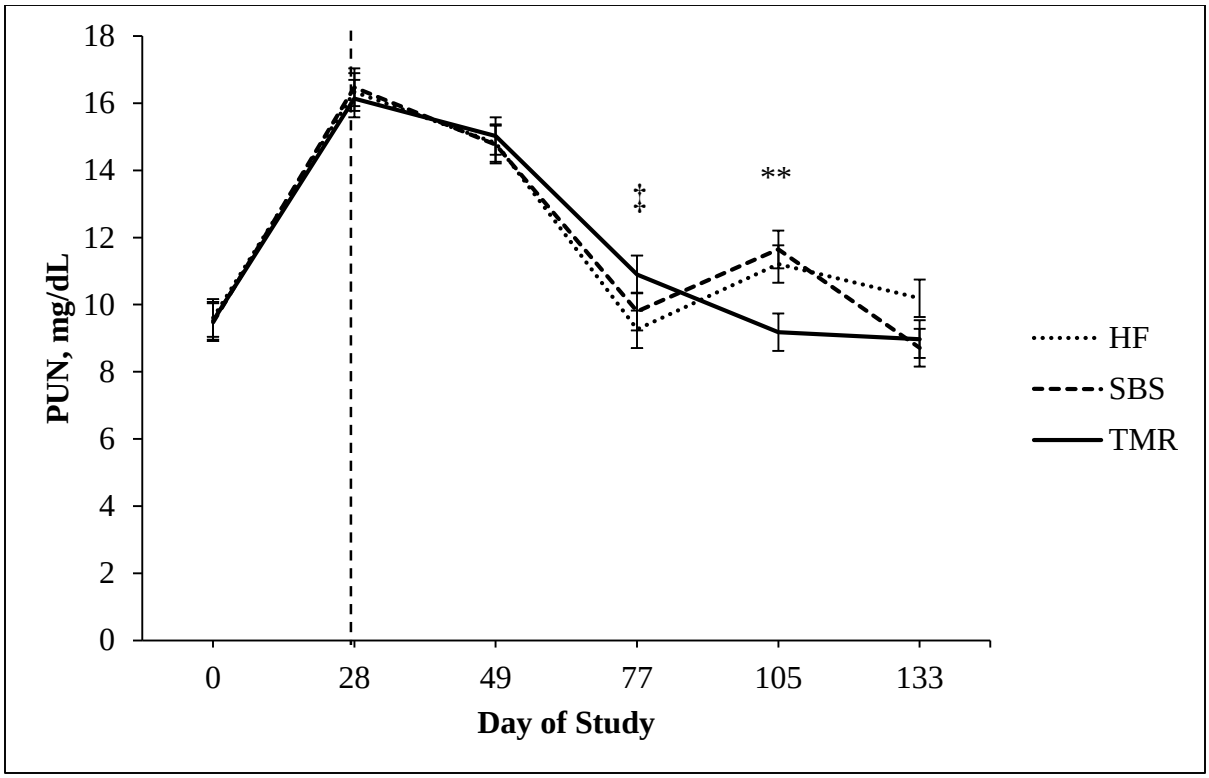

Figure 5.6 Plasma urea nitrogen (PUN) concentrations for heifers fed diets delivered by a hay feeder and feed bunk (HF), side-by-side in a feed bunk (SBS), or a total mixed ration (TMR). Vertical dashed line indicates a switch to a 56:44 forage-to-concentrate diet (from a 40:60) with same feed delivery treatments. A treatment×time interaction was observed ($P < 0.01$). ‡$P < 0.10$; **$P < 0.01$.

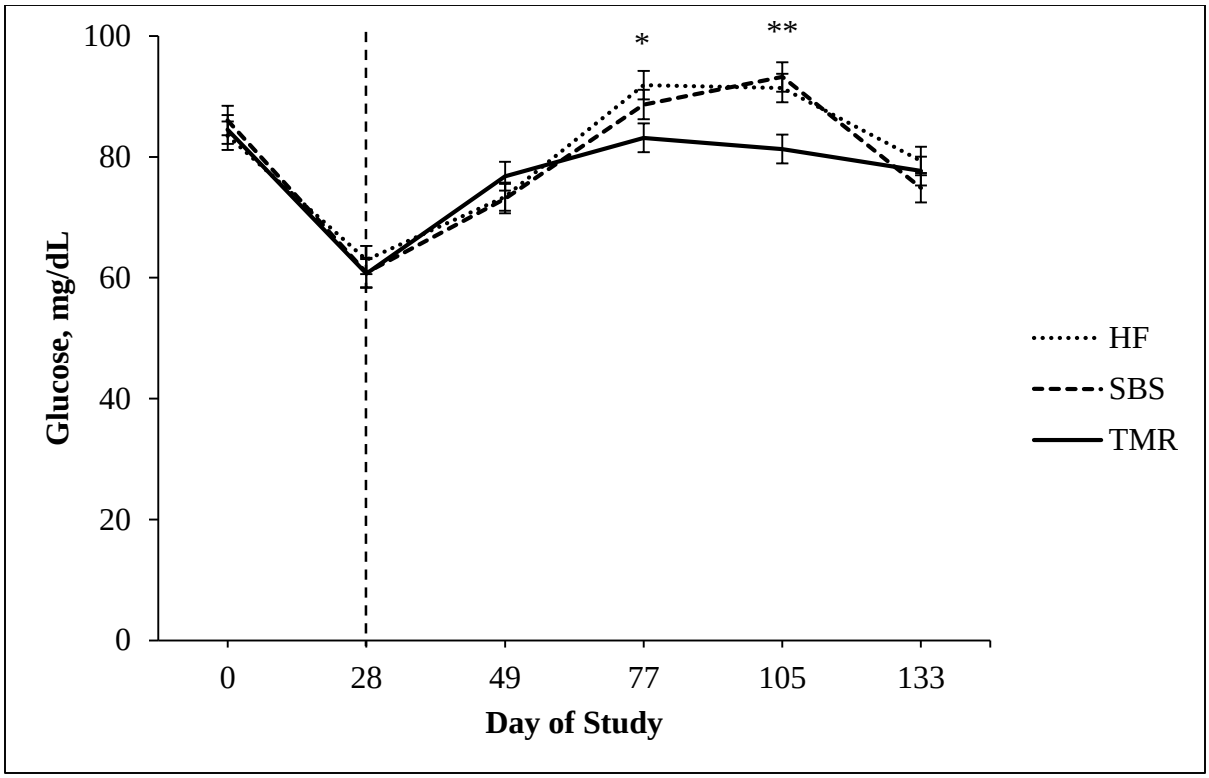

Figure 5.7 Plasma glucose concentrations for heifers fed diets delivered using a hay feeder and feed bunk (HF), side-by-side in a feed bunk (SBS), or a total mixed ration (TMR).Vertical dashed line indicates a switch to a 56:44 forage-to-concentrate diet (from a 40:60) with same delivery treatments. A treatment×time interaction was observed ($P < 0.01$). *$P < 0.05$; **$P < 0.01$.

CHAPTER 6. EFFECTS OF FEEDING HAY AND BALEAGE TO PREPUBERTAL DAIRY HEIFERS DURING THE GROWER PERIOD

6.1 Abstract

Ensiled and dry forages are commonly included in growing dairy heifer diets, yet little research has compared the effects of feeding these different types of forages on dairy heifer performance. The objective of this study was to evaluate effects of feeding baleage to dairy heifers on growth, feed efficiency, diet digestibility, and rumen parameters when transitioning to higher forage diets during the growing period. In the 28-d transition period between arrival and the growing period, 60 Holstein heifers (141.9 ± 1.2 kg of BW) were randomly assigned to 1 of 12 pens and fed a 40% forage diet (DM basis) containing either hay or baleage as the only forage source. Apparent digestibility of the diets was determined using 12 individually-fed heifers. In the 56-d growing period, 36 heifers from the transition period remained on previous treatments and were fed a 60% forage diet (DM basis) with the same forages. In the transition period, ADG was greater for hay than for baleage; however, DMI and feed efficiency were similar between treatments. Plasma urea N was greater for hay on d 14 and 28 and rumen NH3 was greater for hay on d 14. Apparent DM, NDF, and CP digestibility was similar among treatments. In the growing period, heifers fed hay were 6.7 kg heavier than heifers fed baleage at the conclusion of the study. Heifers fed hay consumed more DM and tended

to have greater feed efficiency than heifers fed baleage. Diets containing hay resulted in greater ADG during both the transition and growing periods.

6.2 Introduction

Improving dairy heifer feed efficiency by utilizing highly digestible forage sources can reduce feed and rearing costs. Even though greater quality forage is often more expensive, improvement in forage usage may result in a more cost-effective forage program for developing replacement heifers. Forage inclusion in growing heifer diets is usually high, yet high forage diets are usually poorly digestible. Ensiled forages are commonly used as the primary forage component in heifer diets, although growth and intake responses when feeding ensiled forages as compared to hay are limited and inconsistent. Previous research has shown that feeding alfalfa silage to growing dairy heifers resulted in reduced intakes and weight gain compared to feeding alfalfa hay (Thomas et al., 1961), although the mechanism by which intake and performance were depressed in growing heifers was not elucidated. More recently, when comparing silage to hay for growing cattle, Petit and Flipot (1992a) observed that steers fed an all-silage diet consumed less DM than steers fed an all-hay diet and had greater feed conversions than steers fed an all-hay diet. Also, Petit and Flipot (1992b) found that N and ADF digestibility was increased for steers fed an all-silage diet compared with an all-hay diet.

The use of stretch-wrapping round bales with plastic to produce baleage has become a common practice in recent years. Opportunities exist for heifer growing operations to utilize high quality forages preserved as baleage. Preserving forages as wrapped baleage can increase the number of forage harvests (Savoie and Marcoux,

1985), as well as decrease costs associated with field labor, maintaining harvest machinery (Savoie and Marcoux, 1985) and storage losses (Savoie and Marcoux, 1985; Collins et al., 1987) compared to conventional silage systems on small operations. When evaluating the nutritive value of individually-wrapped baleage for sheep, Beaulieu et al. (1993) observed that ADG and DMI were similar between baleage and conventional chopped silage of similar DM content. Similarly, Charmley and Firth (2004) compared precision-chopped silage with baleage and found that responses in weight gain were greater for yearling steers fed baleage. Other studies have shown that forages ensiled as round bales, when fed to sheep or lactating cows, are more digestible compared to the same forage preserved as hay (Cushnahan and Gordon, 1995; Borreani et al., 2007).

Inconsistency in production responses warrants additional research for feeding baleage. In addition, no work to date has investigated feeding baleage to growing dairy heifers. Therefore, the objective of this study was to evaluate effects of feeding baleage to dairy heifers on growth, feed efficiency, diet digestibility, and rumen fermentation characteristics when transitioning to higher forage diets during the growing period.

6.3 Materials and Methods

Feeding trials were conducted at the Southern Indiana Purdue Agricultural Center (SIPAC) in Dubois, IN from May to September of 2010 using Holstein heifers sourced from raisers within Kentucky Heifer Growers, LLC of Glasgow, KY. All animal-related procedures were conducted in compliance with approved protocols from the Purdue Animal Care and Use Committee (PACUC no. 10-033). The transition period began following arrival at SIPAC, used 12 pens with 5 heifers each in a completely randomized

design, and lasted 28 d. Additionally, a digestibility trial was performed on d 21 to 28 of the transition period using 6 heifers from each treatment. Following the transition period, heifers were slowly adapted to the growing diet for 3 wk prior to the start of the growing period. The growing period used 12 pens with 3 heifers each from the transition period and lasted 56 d. The entire trial lasted 105 d.

6.3.1 Transition Period

6.3.1.1 Animals

Sixty prepubertal heifers (139 ± 10 d of age and 141.9 ± 28.1 kg of BW) were weighed on 2 consecutive d following arrival at SIPAC, stratified by BW, and assigned to 1 of 12 pens with 5 heifers per pen. Pens were then randomly assigned to treatment diets containing either dry hay or baleage as the only forage source. Housing consisted of a naturally ventilated barn with 3.7 m × 21.9 m pens, 3.7 m of feed bunk space, and unrestricted access to water. Pens were covered mid-way by slanted steel roofing and bedded with sawdust throughout the period.

6.3.1.2 Dietary Treatments

Ingredient and nutrient composition of experimental diets are presented in Table 6.1. Forages used were harvested from fields with similar forage species and were selected based on similarities in analyzed nutrient composition in order to reduce forage quality variation. Diets were formulated to allow 0.90 kg/d of ADG and feed was offered at approximately 2.8% of the average pen BW to allow for ad libitum intake and were adjusted daily to minimize refusals. The hay was harvested at SIPAC in 2008 from a second cutting of Kentucky-31 tall fescue (*Festuca arundinacea*) and red clover

(*Trifolium pratense* L.). The baleage was harvested and ensiled at SIPAC in 2009 from a first cutting of the same forage type as the hay. Hay and baleage were baled using a John Deere 567 round baler (Deere and Co., Moline, IL) and baleage was wrapped with 8 to 10 layers of Sun Film (Ambraco, Dubuque, IA) using an Anderson RB9000 in-line bale wrapper (Anderson Group Co., Chesterville, QC, Canada). Hay bales were stored in an enclosed steel-sided barn and in-line wrapped bales were stored outside immediately following harvest. Diets were formulated to be isocaloric and isonitrogenous and contained 40% forage and 60% concentrate on a DM basis. Particle length of forages was reduced using a vertical TMR mixer (Jay-Lor 4575; Jay-Lor Fabricating, Ontario, Canada) prior to mixing treatment diets. The DM of forages was determined every 2 wk to adjust for moisture content. Feed was mixed as a TMR and delivered once per day at 0700 h. Orts were weighed and sub-sampled once per wk when heifers were measured during data collection and feed ingredients were sampled once per month. Feed ingredients and orts were dried at 60°C in a forced air oven, ground through a 1.0 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ), composited by treatment each month, and analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Labs, Ithaca, NY). Samples were analyzed for CP (AOAC 984.13, AOAC, 1990), NDF (addition of heat-treated α-amylase and sodium sulfite; Van Soest et al., 1991), ADF (AOAC 973.18, AOAC, 1990), ME (calculated from TDN in feed; NRC, 2001), and minerals (microwave digestion followed by inductively coupled plasma spectrometry; Isaac and Johnson, 1985).

6.3.1.3 Data Collection and Analysis

Heifers were weighed weekly and skeletal growth measurements, including withers height (WH), hip height (HH), and heart girth circumference (HGC) were taken every 2 wk using a height stick and flexible tape measure, respectively. Body condition score (BCS) was assessed every 2 wk on a scale of 1 to 5 (1 = emaciated, 5 = obese; Edmonson et al., 1989) by 2 evaluators and averaged. Blood samples (10 mL) were collected via jugular venipuncture every 2 wk into vacutainer tubes containing lithium heparin. Plasma was aspirated following centrifugation (2500 x *g* for 15 min at 4°C) and frozen at -20°C for later analysis. Plasma was analyzed for plasma urea N (PUN; procedure no. 0580; Stanbio Laboratory Inc., San Antonio, TX) and glucose (procedure no. 1070; Stanbio Laboratory Inc.). Rumen fluid was obtained on d 0, 14, and 28 using an esophageal tube from the same 2 heifers in each pen and analyzed for pH, VFA, rumen NH3, and cellulose disappearance. Briefly, a speculum was inserted into the mouth and a narrow tube (16 mm external diameter) with a stainless-steel filter on the end (16 mm external diameter) was inserted down the esophagus into the rumen. Vacuum suction was applied using a 60 mL catheter-tip syringe to extract rumen fluid. Rumen fluid was extracted and discarded until saliva was no longer evident in the sample. Then, the first 40 mL of extracted fluid was discarded to further avoid saliva contamination and 60 mL of fluid was collected and saved. Immediately following collection, 8 mL of rumen fluid was placed directly into an 8-mL glass vial and sealed to ensure minimal exposure to O_2 for anaerobic cellulose disappearance. The pH of the remaining rumen fluid was immediately analyzed (model HI 98130; Hanna Instruments, Ann Arbor, MI), and two 20-mL samples of rumen fluid were acidified using 1.0 M

H_2SO_4 (2 mL of acid to 20 mL of sample) and frozen at -20°C for later analysis. Samples were analyzed for VFA using gas chromatography on a bonded capillary column (Supelco, Bellefonte, PA; Erwin et al., 1961) and for NH3 using the Kjeldahl procedure without H_2SO_4 digestion (FOSS Kjeltec 2300, Hoganas, Sweden; AOAC 2001.11). Anaerobic serum tubes (Chemglass Life Sciences, Vineland, NJ) containing 9.0 mL of basal cellulose media [20% clarified rumen fluid; 20% ball-milled cellulose solution (2.0 g Whatman #1 filter paper per 100 mL distilled H_2O); 4.7% Mineral 1 (0.6% KH_2PO_4 w/v); 4.7% Mineral 2 (1.2% NaCl, 0.6% KH_2PO_4, 0.6% $(NH_4)_2SO_4$, 0.25% $MgSO_4 \cdot 7H_2O$, and 0.16% CaCl w/v); 0.4% Na_2CO_3, 0.1% resazurin solution (0.1% w/v); 0.05% L-cysteine HCl; and 50.05% distilled H_2O; anaerobic technique per Bryant and Burkey, 1953 and as modified by Grubb and Dehority, 1976] were inoculated with 1.0 mL of rumen fluid from each heifer. Rumen fluid samples were directly aspirated from the sample vials immediately following sample collection. Samples were serially diluted to 10^{-9} dilution and all tubes were incubated at 37°C for 72 h. Following incubation, total gas volume was measured and tubes were autoclaved at 125°C for 20 min to cease bacterial digestion. After autoclaving, residual cellulose was processed using a micro-NDF procedure described by Pell and Schofield (1993).

6.3.1.4 Digestibility Analysis

One heifer from each pen (n = 6 per treatment) was randomly selected for use in a digestibility trial performed during the transition period. Heifers were individually housed in 2.5 × 2.5 m pens bedded with sawdust from d 24 to 31 relative to the beginning of the transition period. Diets were hand-mixed daily at 0700 h and were delivered to heifers by 0900 h for 8 d. Diets were identical to those used in the transition period, with

the exception that the grower pellet included 0.37% chromic oxide on a DM basis for use as an indigestible external marker. The initial 5 d of the experiment were an acclimation period to housing and diet delivery and was used to ensure a constant DM and chromic oxide intake prior to fecal collection. Approximately 200 g of feces was collected from each heifer every 6 h from d 29 to 31 (12 fecal samples/heifer). Fecal samples were freeze-dried (VirTis 36DX66; SP Scientific, Gardiner NY), ground through a 1.0 mm sieve in a Restch centrifugal mill (Verder International, Vleuten, The Netherlands), and pooled and analyzed by heifer for CP (FOSS Kjeltec 2300; AOAC 984.13, AOAC, 1990), NDF (Ankom A200 fiber bag technique; Van Soest et al., 1991), and chromic oxide content (Williams et al., 1962). The concentration of chromic oxide in the feed and feces was used to calculate apparent DM, CP, and NDF digestibility of each diet (McGuire et al., 1966).

6.3.2 Grower Period

6.3.2.1 Animals

Thirty-six heifers (189 ± 9 d of age and 185.6 ± 26.6 kg of BW) utilized in the transition period remained on previous dietary treatments in the same housing and pens for the growing period. Two heifers from each pen were removed for use in a concurrent study following the transition period. Heifers were removed based on BW to ensure even distribution of weights across pens and treatments at the start of the adaptation for the growing period. During the growing period, one heifer was euthanized due to a fractured femur and one heifer died due to respiratory illness unrelated to treatments. These heifers

were in the same pen and therefore one pen fed the hay treatment was removed from analysis.

6.3.2.2 Dietary Treatments

Ingredient and nutrient composition of experimental diets are presented in Table 6.1. Forages used were harvested from the same fields in order to reduce forage composition variation. Diets were formulated to allow 0.90 kg/d of ADG and feed was offered at approximately 2.8% of the average pen BW to allow for ad libitum intake and were adjusted daily to minimize refusals. The hay was harvested at SIPAC in 2010 from a first cutting of low endophyte-infected tall fescue (*Festuca arundinacea* L. Schreb) and red clover (*Trifolium pratense* L.) forage. The baleage was harvested and ensiled at SIPAC in 2010 from a first cutting of the same forage type as the hay. The same equipment outlined in the transition period was used to bale and wrap the forage used in the growing period. Hay and wrapped bales were stored as described in the transition period. Diets were formulated to be isocaloric and isonitrogenous and contained 60% forage and 40% concentrate on a DM basis. Following the transition period, heifers were allowed to adjust to their new diets for 14 d prior to the start of data collection. Forages were processed as described previously. Feed was mixed as a TMR and delivered once per d at 0700 h. Orts were weighed and sub-sampled monthly when heifers were measured during data collection and feed ingredients were sampled once per month. Feed ingredients and orts were dried at 60°C in a forced air oven, ground through a 1.0 mm screen using a Wiley mill (Thomas Scientific), composited by treatment on a monthly basis, and analyzed for nutrient composition by a commercial laboratory (Dairy

One Forage Labs). Samples were analyzed for CP, NDF, ADF, ME, and minerals as described previously.

6.3.2.3 Data Collection and Analysis

Heifers were weighed every 2 wk and WH, HH, HGC, and BCS were taken monthly as described previously. Blood samples (10 mL) were collected via jugular venipuncture monthly and analyzed for PUN and glucose as described earlier. Rumen fluid was obtained using an esophageal tube on d -14 and 56 from the same 2 heifers in each pen and analyzed for pH, VFA, rumen NH3, and cellulose disappearance as described previously.

6.3.3 Statistical Analysis

Each period was analyzed separately and means are reported for each period. Pens were assigned to treatments in a completely randomized design, with heifers randomly assigned by BW to pens. Data, excluding digestibility and cellulose disappearance, were analyzed as repeated measures (Littell et al., 1998) using the MIXED procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The variance-covariance matrix structures were evaluated for each model using simple, first order auto-regressive, compound symmetry, and unstructured covariance structures. Variance-covariance matrix structures were selected for each model based on lowest Bayesian information criterion fit statistic. Treatment, time, and the interaction of the two variables were included in the model as fixed effects and starting PUN and glucose concentrations were included in respective models as covariates in the grower period. Cellulose disappearance and gas production were analyzed as a single

measurement at the conclusion of each period. Means reported for cellulose disappearance and gas production are from the highest dilution with a significant difference for the response variable (10^{-8} in each instance). For digestibility analysis, heifer was considered the experimental unit because heifers were individually fed and DMI and chromic oxide concentrations were known. Digestibility, cellulose disappearance, and gas production models included treatment as a fixed effect and heifer nested within treatment as a random effect.

6.4 Results and Discussion

6.4.1 Transition Period

Heifer growth measurements for the transition period are presented in Tables 6.2 and 6.3. Heifer BW was similar between treatments, averaging 168.1 kg at the conclusion of the period ($P = 0.26$). Average daily gains were significantly different between treatments, with heifers fed hay gaining more weight per day than heifers fed baleage ($P < 0.05$). Dry matter intake was similar between treatments ($P > 0.10$), averaging 5.0 kg/d. Additionally, NDF intake, CP intake, and feed efficiency were similar among treatments. Similar feed efficiency between treatments was unexpected given the significant advantage in ADG for hay, but is likely due to variation within pens during the period. Hip and withers heights and HGC were similar among treatments across the period ($P > 0.10$), averaging 110.6, 104.8, and 127.0 cm at the conclusion of the transition period, respectively. Body condition scores and the changes in heights and HGC from the start to the end of the measurement period were also similar among treatments, an expected result given the short duration (4 wk) of the transition period.

At the beginning of the transition period, PUN and glucose were similar between treatments (Table 6.4). Heifers fed hay had greater PUN concentrations on d 14 and d 28 compared to heifers fed baleage. Additionally, PUN for heifers fed baleage did not increase until d 28, whereas heifers fed hay had greater PUN concentrations on d 14. Glucose tended to be greater on d 14 for heifers fed hay compared to heifers fed baleage, but glucose significantly declined for both treatments across the period. Concentrations of PUN observed during the transition period correspond to normal PUN concentrations observed for growing beef cattle (Byers and Moxon, 1980). It is likely that the increased PUN for hay was due to increased availability of protein in the hay diet compared to the baleage diet. Verbic et al. (1999) found that microbial CP supply was greatest for hay compared to direct cut and wilted silages fed to sheep, which may explain the differences in ADG seen in the current trial. Verbic et al. (1999) also found that synchrony of protein and organic matter degradation was more favorable for hay compared to silages. However, Petit and Flipot (1992b) observed that steers fed timothy silage as the sole forage source had significantly greater PUN concentrations compared to steers fed timothy hay, in contrast to the values observed in this study. Since NDF and CP intake was similar between treatments, heifers fed hay may have utilized energy and protein in the diet more efficiently for weight gain than heifers fed baleage. Additionally, the ratio of ADG to daily NDF intake was similar between treatments in the transition period, indicating that heifers fed hay were more efficient at utilizing NDF in the diet compared to heifers fed baleage. In contrast, Petit and Flipot (1992b) found that when growing steers were fed an all-forage diet as either grass hay or silage, N and ADF digestibility was increased for steers fed silage. Cushnahan and Gordon (1995) observed that when

forage was ensiled as round bales, apparent digestibility of DM and N increased compared to forage preserved as hay when fed to sheep. In the study by Cushnahan and Gordon (1995), however, adverse conditions occurred when hay was harvested, leading to greater quality losses for the hay used in the study. It is unclear as to why the silage used in this study resulted in similar apparent digestibility in contrast to past research, though differences in animal requirements and rumen development may play a role.

Rumen NH3, acetate, propionate, and total VFA were greater at d 14 for hay compared to baleage ($P < 0.05$), but NH3 and VFA concentrations were similar between treatments on d 28. The increase in rumen NH3 and total VFA concentrations early in the transition period indicate greater breakdown of nutrients in the rumen and may explain the improvement in ADG observed for heifers fed hay. It is likely that more microbial CP was synthesized from the hay diet given the increase in fermentation products, which could have contributed to weight gain. Acetate:propionate remained constant throughout the trial for heifers fed hay; however, heifers fed baleage had greater acetate:propionate on d 14 compared to heifers fed hay. This may be due to the treatment×time effect observed for acetate and propionate concentrations, where acetate and propionate increased for hay from d 0 to d 14 ($P < 0.05$) but were similar for baleage from d 0 to d 14 ($P > 0.10$). Changes in the proportion of VFA between treatments over time may indicate differences in digestibility and could explain production responses observed in this study, though apparent DM digestibility was not different between treatments ($P = 0.19$; Table 6.5). Cellulose disappearance and total *in vitro* gas production were similar between treatments on d 28 (Table 6.4), averaging 30.8% and 3.8 mL at the highest serial dilution. Time of rumen fluid sampling may have influenced

bacterial activity, as samples were collected prior to feed delivery in the morning. Fiber-digesting bacteria are pH-sensitive and cellulolytic activity increases at pH above 6.0 (Russell and Wilson, 1996). The lowest pH value observed in this period was above 6.0, which should not have inhibited microbial growth in either treatment. However, cellulose disappearance was only determined on d 28 of the period and pH was similar between hay and baleage on d 28, which suggests that bacterial populations were similar at the time of sampling for each treatment.

6.4.2 Grower Period

Heifers used in the grower periods remained on their previous treatment and pens were balanced for BW on d -14 after two heifers from each pen were removed for use in a concurrent study. Heifers were allowed to adapt to their new 60:40 forage-to-concentrate diets for 2 wk. Growth measurements for this period are presented in Tables 6.2 and 6.3. Heifers fed hay were 6.7 kg heavier than heifers fed baleage at the conclusion of the grower period and ADG was greater for H across the period ($P < 0.05$). However, ADG decreased from the transition to the grower period, most likely due to the decrease in energy and protein of the grower diet compared to the transition diet (Table 6.1). Additionally, growing heifers utilize higher concentrate diets more efficiently than higher forage diets, as they retain more and excrete fewer nutrients (Reynolds et al., 1991). Feed intake was greater when heifers were fed hay compared to baleage; however, average daily NDF and CP intake was similar between treatments and ADG per kg of daily NDF intake was greater for heifers fed hay compared to baleage ($P < 0.01$). Heifers fed hay tended to have improved feed efficiency, which may be due to improved

utilization of nutrients in the hay diet. Specifically, weight gains indicated that heifers fed the hay diet had better gains per unit of NDF and CP intake compared to heifers fed baleage. Other studies have observed reductions in DMI when feeding silage (Thomas et al., 1961a; Waldo et al., 1969; Clancy et al., 1977), agreeing with the results of this study. However, Merchen et al. (1986) observed that lambs and steers fed diets containing direct-cut silage, low-moisture silage, or hay had similar DMI, contrary to intakes observed in this study. Additionally, El Serafy et al. (1974) found that steers fed alfalfa-bromegrass hay consumed less DM as a percent of BW than steers fed alfalfa-bromegrass haylage. However, in the study by El Serafy et al. (1974), the forages provided were the sole source of protein and energy for the steers and gut-fill may have influenced DMI. In a growing phase feedlot study by Petit and Flipot (1992a), steers fed an all-silage diet consumed 49% less DM than steers fed an all-hay diet. However, steers fed silage were 1.9 times more feed efficient than steers fed hay (Petit and Flipot, 1992a), contrary to the results observed in the current study. Although not directly measured during the grower period, increased digestibility of the hay diet may explain the improvements in production responses seen in this study. It is still unclear why DMI is depressed when young animals are fed silage as a primary forage source, although Thomas et al. (1961b) hypothesized that decreased forage DM at ensiling and high concentrations of fermentation products in silage may be responsible for decreased intakes in growing heifers. Similar to the transition period, skeletal growth did not differ between treatments. Hip and withers heights and HGC averaged 117.4, 111.8, 139.7 cm across the growing period, respectively. The change in heights and HGC from the start to the end of the measurement period were also similar ($P > 0.10$) and BCS decreased for both

treatments ($P < 0.05$), suggesting that additional ADG for hay was either due to gut-fill or deposited as lean tissue in lieu of skeletal growth or external fat deposition.

Plasma metabolites and rumen fermentation characteristics in the grower period are presented in Table 6.6. Initial PUN concentrations on d -14 were included in the model as a covariate. After the two week acclimation to the grower diet, PUN was greater for hay than for baleage on d 0, but PUN was similar between treatments across the remainder of the period. Blood glucose and rumen NH3 were also similar between treatments, but did decrease from d -14 to d 56 for both treatments ($P < 0.05$). Concentrations of VFA were not influenced by treatment; however, concentrations of acetate and propionate as a percentage of the total VFA concentration significantly increased over time for both treatments and percentage of propionate tended to decrease for heifers fed hay ($P = 0.09$). Additionally, acetate:propionate increased ($P = 0.05$) from d -14 to d 56 for hay. Increased pH and concentrations of organic acids, particularly acetate, were expected due to the increased inclusion of forage in each diet compared to the transition period, though pH was similar in each period. Cellulose disappearance was similar between diets, but declined significantly from d -14 to d 56 ($P < 0.01$). Erfle et al. (1982) found that cellulolytic bacterial counts *in vitro* significantly declined as pH decreased from 6.0 to 5.5 with mixed rumen cultures. Additionally, acetate production increased at pH greater than 6.5 in continuous culture (Erfle et al., 1982), similar to results observed by Slyter et al. (1966), suggesting that in the current study where pH averaged 6.8 across treatments, cellulolytic bacteria populations should have been highly active in both treatment groups. It is unclear as to why *in vitro* cellulose disappearance

declined over time in this study as concentrations of VFA and pH increased on the higher forage diet, which typically indicates more favorable rumen fermentation.

6.5 Summary and Conclusions

Feeding dry hay as a forage source for growing dairy heifers improved ADG and tended to improve feed efficiency in the current study, which may have been a result of improved NDF digestibility of the hay. Greater growth rates when feeding hay agree with previous studies using sheep and lactating cows, but not with growing beef calves, which suggests that more research is needed to determine if physiological state influences utilization of forages preserved as baleage. These data suggest that increases in rumen fermentation products during the transition period may explain improved ADG for heifers fed hay, possibly indicating more favorable rumen adaptation during this period.

Table 6.1 Ingredient and analyzed nutrient composition of forages and experimental diets.

	Transition Period		Grower Period	
Item	Hay	Baleage	Hay	Baleage
Ingredient, % of DM				
Hay	40.0	--	60.0	--
Baleage	--	40.0	--	60.0
Grain mix[1]	60.0	60.0	40.0	40.0
Forage nutrient composition[2]				
DM	92.5	48.1	91.4	44.2
ME, Mcal/kg	2.23	2.22	2.07	1.96
CP	14.6	15.9	11.5	10.3
NDF	54.2	55.6	67.7	70.9
ADF	37.7	40.6	47.8	47.9
Diet nutrient composition[2]				
DM	90.0	67.6	91.2	62.8
ME, Mcal/kg	2.98	2.98	2.62	2.58
NE_g, Mcal/kg	1.20	1.20	0.98	0.93
CP	18.4	18.9	16.5	15.8
NDF	29.2	29.7	46.2	48.2
ADF	18.8	20.0	31.5	31.6
Ca	1.11	1.16	1.20	1.03
P	0.56	0.54	0.50	0.53

[1]Grain mix consisted of 60% dry rolled corn and 40% commercial grower pellet (3.58 Mcal/kg ME, 38.6% CP, 17.9% NDF, 10.2% ADF, 3.1% Ca, and 1.4% P on a DM basis) in the transition period and 42.5% dry rolled corn and 57.5% commercial grower pellet in the grower period on a DM basis.

[2]All values given as a percent of DM unless otherwise stated.

Table 6.2 Effects of feeding dry hay or baleage to prepubertal dairy heifers on body weight, average daily gain (ADG), dry matter intake (DMI), and feed efficiency in the transition and grower periods.

Item	Hay	Baleage	SEM	*P*-value
Transition period				
Initial BW, kg	141.8	142.0	1.81	0.93
Final BW, kg	170.0	167.0	1.81	0.26
ADG, kg/d	1.01	0.89	0.04	0.04
DMI, kg/d	4.97	5.06	0.076	0.44
CP intake, kg/d	0.94	0.96	0.014	0.22
NDF intake, kg/d	1.45	1.50	0.022	0.14
Feed efficiency[1]	0.205	0.178	0.012	0.14
NDF conversion[2]	0.702	0.601	0.027	0.03
Grower period				
Final BW, kg	218.7	212.0	1.98	0.02
ADG, kg/d	0.63	0.55	0.02	0.01
DMI, kg/d	5.69	5.40	0.067	< 0.01
CP intake, kg/d	0.90	0.89	0.005	0.30
NDF intake, kg/d	2.62	2.59	0.016	0.25
Feed efficiency[1]	0.113	0.107	0.002	0.06
NDF conversion[2]	0.245	0.222	0.004	< 0.01

[1]Feed efficiency was calculated as the ratio of ADG to DMI.
[2]NDF conversion was calculated as the ratio of ADG to NDF intake.

Table 6.3 Effects of feeding dry hay or baleage to prepubertal dairy heifers on skeletal growth and body condition score (BCS) in the transition and grower periods.

Item	Hay	Baleage	SEM	*P*-value
Transition period				
Hip height, cm				
Initial	106.2	106.5	0.30	0.50
Final	110.8	110.6	0.30	0.59
Change	4.7	4.0	0.30	0.13
Withers height, cm				
Initial	99.8	99.7	0.36	0.79
Final	104.8	104.6	0.36	0.65
Change	5.0	4.9	0.31	0.82
Heart girth, cm				
Initial	122.8	122.5	0.60	0.74
Final	126.8	127.1	0.60	0.69
Change	4.0	4.6	0.43	0.33
BCS, 1 to 5 scale				
Initial	2.90	2.88	0.02	0.49
Final	2.87	2.82	0.02	0.11
Change	-0.04	-0.07	0.03	0.43
Grower period				
Hip height, cm				
Initial	110.5	110.5	0.61	0.99
Final	117.1	117.6	0.64	0.50
Change	6.3	7.3	0.46	0.11
Withers height, cm				
Initial	104.4	104.6	0.56	0.81
Final	112.0	111.5	0.58	0.49
Change	7.5	6.7	0.52	0.31
Heart girth, cm				
Initial	127.0	127.3	1.09	0.96
Final	140.2	139.2	1.14	0.53
Change	12.5	12.1	0.80	0.68
BCS, 1 to 5 scale				
Initial[1]	2.89	2.83	0.02	0.10
Final	2.64	2.59	0.02	0.15
Change	-0.24	-0.24	0.03	0.97

[1]Intial BCS in the grower period was included in the model as a covariate.

Table 6.4 Effects of feeding dry hay or baleage to prepubertal dairy heifers on blood metabolites and rumen fermentation characteristics in the transition period.

	Hay			Baleage				*P*-value[1]		
Item	0 d	14 d	28 d	0 d	14 d	28 d	SEM	T	D	T x D
PUN[2], mg/dL	9.3	12.0	16.4	10.1	10.0	14.6	0.43	0.02	<0.01	<0.01
Glucose, mg/dL	93.2	83.3	66.1	91.1	79.1	66.5	1.68	0.18	<0.01	0.42
pH	6.99	6.58	6.68	6.97	6.85	6.58	0.08	0.43	<0.01	0.03
Rumen NH3, mg/dL	11.3	15.5	17.0	12.3	11.7	17.0	0.90	0.31	<0.01	<0.01
Total VFA[3], mmol/L	48.4	65.2	48.9	42.2	43.4	57.7	5.22	0.27	0.04	<0.01
VFA[4], %										
Acetate	69.3	68.8	71.7	70.0	75.6	72.3	1.42	0.07	0.06	<0.01
Propionate	19.9	21.4	18.5	20.6	15.5	17.9	1.18	0.13	0.08	<0.01
Butyrate	7.2	5.4	5.3	6.1	4.8	5.5	0.43	0.32	<0.01	0.13
Acetate:propionate	3.6	3.5	4.0	3.5	5.1	4.2	0.43	0.09	<0.01	<0.01
Cellulose disappearance, %	--	--	37.4	--	--	24.1	7.4	0.22	--	--
Total gas production, mL	--	--	4.3	--	--	3.3	0.59	0.22	--	--

[1]T = treatment; D = day; T x D = treatment by day interaction.
[2]Plasma urea nitrogen.
[3]Volatile fatty acid.
[4]Values given as a percent of the total VFA concentration.

Table 6.5 Apparent digestibility of diets containing either dry hay or baleage fed to individual prepubertal dairy heifers (n = 12).

Item	Hay	Baleage	SEM	*P* value
Initial BW, kg	167.1	165.5	1.67	0.52
Final BW, kg	167.9	166.7	1.95	0.69
DMI, kg/d	4.8	4.7	0.10	0.44
Apparent digestibility				
DM, %	68.4	66.6	0.88	0.19
NDF, % of DM	68.1	65.4	1.33	0.19
CP, % of DM	64.2	62.2	0.95	0.17

Table 6.6 Effects of feeding dry hay or baleage to prepubertal dairy heifers on blood metabolites and rumen fermentation characteristics in the grower period.

	Hay		Baleage			*P* value[1]		
Item	-14 d	56 d	-14 d	56 d	SEM	T	D	T x D
PUN[2,3] , mg/dL	15.4	13.9	14.7	13.6	0.44	0.38	<0.01	0.07
Glucose[3], mg/dL	65.8	53.7	63.4	55.3	1.54	0.79	<0.01	0.19
pH	6.68	6.96	6.58	6.88	0.06	0.14	<0.01	0.84
Rumen NH3, mg/dL	16.4	12.9	16.2	13.5	1.00	0.85	<0.01	0.56
Total VFA[4], mmol/L	46.1	57.8	55.1	60.4	6.24	0.47	0.06	0.46
VFA[5], %								
Acetate	71.1	73.9	72.4	74.1	0.87	0.45	<0.01	0.44
Propionate	18.5	16.6	18.2	18.0	0.73	0.56	0.05	0.09
Butyrate	5.5	5.8	5.4	5.5	0.27	0.51	0.34	0.56
Acetate:propionate	3.9	4.5	4.1	4.2	0.19	0.87	0.02	0.05
Cellulose disappearance, %	37.0	4.8	24.1	0.0	5.55	0.15	<0.01	0.36
Total gas production, mL	4.4	0.1	3.2	0.1	0.39	0.22	<0.01	0.06

[1]T = treatment; D = day; T x D = treatment by day interaction.
[2]Plasma urea nitrogen.
[3]Initial concentration on d -14 included as a covariate.
[4]Volatile fatty acid.
[5]Values given as a percent of the total VFA concentration.

CHAPTER 7. OVERALL SUMMARY AND IMPLICATIONS

Alternative feeding strategies and management practices that maintain replacement heifer growth and performance while reducing costs are appealing options for dairy producers to remain economically sustainable. Studies outlined in this dissertation aimed to address feeding practices commonly encountered in the industry for replacement heifers and their effects on growth and development. Knowledge gleaned from the previously discussed research will help improve nutritional recommendations for weaned replacement heifers, a stage of dairy production that is often overlooked.

In Study 1 and 2, we evaluated low versus high NFC post-weaning diets for dairy calves 3 to 8 mo of age. Study 1 (Chapter 2) evaluated the potential effects of pre-weaning diet on performance post-weaning when NFC was altered in post-weaning diets for 3 to 7 mo-old heifers and steers. Study 2 (Chapter 3) focused on comparing NFC concentrations in the diet in addition to source of ME to determine how carbohydrate and energy availability affect growth in 4 to 8 mo-old heifers. High starch carbohydrate sources are typically more digestible when compared to high fiber carbohydrate sources and are differentially fermented in the rumen, resulting in altered VFA profiles. Fermentation favoring propionate and butyrate production is associated with improved energy utilization, efficiency, and rumen development in younger ruminants; in contrast, fermentation favoring acetate is often considered less efficient from an energy standpoint, though it is important for rumen health and milk fat production lactating dairy cattle. In

Study 1 (Chapter 2), feeding high NFC diets post-weaning resulted in increased growth, efficiency, and altered rumen fermentation profiles in favor of more propionate and butyrate production. Evaluating interactions of pre-weaning and post-weaning nutrition found animals that were fed a high plane of nutrition pre-weaning and a low NFC diet post-weaning were the lightest at 7 mo of age and were less feed efficient in the early post-weaning period. When evaluating rumen development, NFC content did not affect tissue morphology at 28 wk of age; however, it is unclear how NFC may have affected tissue development up to 28 wk of age, particularly when greater proportions of concentrate were fed early in the post-weaning period. Interestingly, pre-weaning nutrition appeared to have some long-term effects on rumen development, as surface area of rumen tissue was increased for 7 mo-old steers fed a conventional compared to a high plane of nutrition pre-weaning. However, this did not result in an increase in performance post-weaning, but may partially explain why animals fed conventional planes of nutrition perform similarly to calves fed high planes of nutrition despite growth advantages for high planes of nutrition pre-weaning. In Study 2 (Chapter 3) however, feeding high NFC diets did not result in improved performance compared to feeding low NFC diets, despite differences in rumen fermentation profile when hay inclusion was 60% of the diet. An interesting observation was in NDF and forage NDF intake, as it appeared that total NDF does not limit DM intake in calves consuming 35% hay diets, but may physically restrict intake when hay is increased to 60% of the diet on a DM basis. This is likely due to the forage NDF inclusion in the diet, as forage NDF intake was restricted to less than 1.0% of BW on a DM basis when hay inclusion in the diet was 60%. Understanding feed intake regulation in young calves aids in predicting intake and

growth, as current prediction equations appear to under-predict intake and growth in weaned calves to 6 mo of age.

In Study 3 (Chapter 4), we sought to determine appropriate inclusion rates of concentrates in the diets of 4 to 8 mo old prepubertal dairy heifers in order to optimize growth and feed efficiency. Typical weaned heifer diets include high concentrations of forages and high-fiber concentrate sources that are associated with reduced growth and efficiency when compared to feeding cereal grains and low-fiber concentrate sources. We observed linear increases in growth when concentrate proportions in the diet increased from 40 to 80% of the diet when fed with dry hay; however, when rapidly switched to a common diet containing 40% concentrate, performance immediately following the switch was significantly reduced for heifers previously consuming an 80% concentrate diet. While we observed an interaction of treatment diet over time following a diet switch for rumen fermentation profiles, samples collected did not capture acute differences relative to a diet change. If I were to repeat this trial, I would schedule rumen fluid collections within the week following the diet switch to determine if differences in rumen fermentation profile correspond to differences in growth and efficiency observed during the study. Additionally, from economic comparisons of feed costs, we determined that feeding moderate concentrate diets (60%) to younger calves was less expensive per kg of BW gain compared to feeding a lower concentrate diet. This has significant cost-saving implications, particularly for larger farms and heifer enterprises, as days on feed would be reduced as a result of greater weight gains early in the grower period, resulting in an opportunity to reduce age at first calving.

In Study 4 (Chapter 5), feed delivery methods were evaluated to discern appropriate feed management recommendations for 4 to 8 mo old heifers. Heifers are typically fed using a TMR on larger heifer raising operations or large dairies that raise their own replacement heifers as consistent supplies of nutrients are provided and feed sorting is discouraged. Alternatively, component-feeding is often used in lieu of purchasing expensive mixing equipment on smaller farms. In our study, heifers fed using a hay feeder with grain fed separately or hay and grain fed side-by-side in a bunk grew faster and were more feed efficient when offered a 40% hay diet compared to heifers fed using a TMR. Heifers fed using a hay feeder were also heaviest at the conclusion of the study due to increased average daily gain, but feed efficiency was similar among feed delivery methods when hay inclusion increased to 56% of the diet. Additionally, variation in BW increased for heifers fed using a hay feeder, which may have potential to introduce variation in growth rates with component feeding compared to using a TMR. Blood metabolites and rumen fermentation profiles were also similar among feed delivery methods; however, the time relative to feeding in which these samples were collected may limit our understanding of energy utilization due to feed delivery method. As blood and rumen fluid samples were taken immediately before feeding, diurnal variation in rumen fermentation due to meal size and duration after feeding was not captured. Though the diet delivered was identical across treatments, heifers fed using a hay feeder increased voluntary DM intake when hay increased in the diet, which would have altered the diet consumed compared to heifers fed using the TMR. Therefore, provision of diet components separately in may be more appropriate earlier in the grower

period for prepubertal dairy heifers to enhance performance, and switching to TMR-feeding may need to be later than what is conventionally seen in the industry.

In Study 5 (Chapter 6), evaluation of preserved forages sought to identify the optimal forage type to include in diets for weaned heifers from 4 to 8 mo of age. Forages are inexpensive sources of energy for ruminants and are often included at high rates in diets of growing heifers. However, intake and digestibility of forages fed to growing heifers can influence growth and efficiency. When heifers were fed baleage at 40% of the diet, ADG was reduced compared to heifers fed the same forage preserved as dry hay. Increasing forage inclusion to 60% of the diet resulted in a 12.5% improvement in ADG and a 6.7 kg advantage in BW at 7.5 mo of age for heifers fed hay compared to baleage. Much of this response was due to reduced intakes for heifers fed baleage combined with a tendency for an improvement in feed efficiency for heifers fed hay. Rumen fermentation profiles alluded to more favorable diet utilization for heifers fed hay at lower dietary inclusion, suggesting differences in diet digestibility immediately following a diet change. However, diet digestibility was similar between forage preservation methods, despite a numerical increase in NDF digestibility observed in heifers fed hay. One major limitation to this study was the lack of digestibility measurements over time during the first 28 d to coincide with rumen fermentation measurements as well as digestibility measurements after forage inclusion was increased to 60% of the diet. The mechanisms by which intake declines when fermented forages are fed is still unclear, and future research should aim to compare other dry and fermented forages commonly utilized in heifer diets.

Overall, data from this dissertation should be used to aid in designing feeding programs for weaned, prepubertal heifers in order to optimize growth and efficiency. Given the data presented herein, it is recommended to:

1. Consider pre-weaning nutrition when formulating weaned heifer diets in order to optimize and ensure consistent growth from birth to puberty.
2. Consider energy sources (fiber vs. starch vs. fat) when formulating growing heifer diets to optimize growth pre-puberty.
3. Include concentrates in weaned heifer diets from 60 to 65% of the diet DM then gradually increase forage inclusion thereafter to optimize growth and minimize feed costs per kg of BW gain.
4. Intake in heifers appears to be differentially regulated according to the amount of forage included in the diet, as calves can consume more DM to meet energy requirements when total NDF is high and forage inclusion is low, but intake can be restricted when both total NDF and forage inclusion is high (>50% of diet on a DM basis).
5. Use component-feeding management for younger weaned heifers and introduce a TMR later in the prepubertal growing period (after 6 mo of age).
6. Avoid fermented forages when feeding heifers under 6 mo of age.

Better understanding of the dynamic changes that occur pre- to post-weaning in dairy heifers is still required, as gaps in knowledge exist with respect to digestive physiology development around the time of weaning and thereafter. However, the data presented herein illustrates the need to account for several factors, including feed management and nutrient composition, in order to develop a successful prepubertal heifer feeding program.

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VITA

VITA

Tana S. Dennis

Education

Doctor of Philosophy 08/2016
Purdue University, West Lafayette, IN
Concentration: Dairy Cattle Nutrition, Rumen Development, and Management
Advisor: Drs. Tamilee Nennich and Jon Schoonmaker

Masters of Science 05/2011
Purdue University, West Lafayette, IN
Concentration: Dairy Cattle Nutrition and Management
Advisor: Dr. Tamilee Nennich

Bachelors of Science 05/2008
University of Florida, Gainesville, FL
Major: Animal Sciences
Concentration: Animal Biology

Research Experience

Doctor of Philosophy Research Program 06/2011 to 08/2016
Proposed Dissertation Title: INFLUENCE OF DIET MANIPULATION AND FEED MANAGEMENT STRATEGIES ON GROWTH AND RUMEN DEVELOPMENT OF POST-WEANED DAIRY HEIFERS

- Overall laboratory objectives investigated dairy replacement heifer nutrition and management, co-product feeding for dairy cattle, and whole-farm nutrient management
- Personal research accomplishments:
    - Investigated feeding value of tall fescue round bale silage as compared to hay for growing dairy heifers
    - Evaluated feed delivery strategies for dairy heifers
    - Researched the influences of diet manipulation, including altering forage and grain proportions and dietary carbohydrates, on growth and feed efficiency of dairy heifers
    - Explored rumen development in dairy heifers from post-weaning to 6 months of age
    - Determined economic impacts of different feed management regimes
- Managed research laboratory including overseeing employees, developing and implementing standard operating procedures, and managing laboratory equipment and inventory

- Mentored undergraduate students in the laboratory and helped manage and execute undergraduate research projects
- Conducted research projects on campus, at university research centers, and on commercial dairy operations

Masters of Science Research Program 08/2008 to 05/2011
Thesis: EFFECTS OF MIXED GRAZING WITH GOATS AND CO-PRODUCT SUPPLEMENTATION ON DAIRY HEIFER PERFORMANCE AND PASTURE COMPOSITION

- Personal research accomplishments:
  - Investigated efficacy of supplementing dairy heifers with soybean hulls and dried distiller's grains on mixed species pasture
  - Identified grazing strategies to improve forage quality in rotationally-stocked pastures
  - Evaluated the impact of mixed livestock species grazing on animal performance
  - Provided dairy producers with practical recommendations for raising replacement heifers on pasture
- Managed research laboratory and 1 to 2 undergraduate student employees each semester
- Mentored undergraduate students in the laboratory and helped manage and execute undergraduate research projects

Teaching Experience

Dairy Farm Management (ANSC 44400) Spring/Fall 2012, 2013, 2014

- Teaching assistant for production course with semester enrollment from 15 to 40 students
- Lectured on topics including calf and heifer management, mammary development and physiology, general dairy cattle nutrition, micronutrient nutrition, and feed management
- Instructed hands-on labs at the Purdue Dairy Research Center covering topics including milking procedures, feed management, and neonatal calf management
- Responsible for providing exam questions and keys, grading exams, and written homework
- Facilitated dairy farm visits for students to ask producers questions and record virtual farm tours as a teaching aid for on-farm problem solving

Applied Animal Nutrition (ANSC 32400) Spring 2009, 2013

- Teaching assistant responsible for instructing laboratory section of 30 students each semester
- Lectured on applied nutrition concepts related to calculating nutrient content of feeds and livestock rations and balancing rations using Microsoft Excel and BRILL computer programs
- Managed broiler chick experiment in which students were responsible for designing and mixing experimental diets, feeding and watering chicks, and collecting growth data
- Guided students when designing experimental diets and analyzing experimental data
- Responsible for grading laboratory homework and in-class exams

Extension and Service Activities

Youth Development

- National FFA Milk Quality Career Development Event Judge (2012)
- Gibson County (Indiana) Seventh Grade Science Sensations Cow Demo (2011, 2013)
- 4-H and FFA Dairy Foods Judging Competition Volunteer (2010, 2011, 2012, 2013, 2014)
- 4-H Young Dairy Producer Competition Volunteer (2009, 2010, 2011, 2014)

Departmental Service

- ANSC 18100 Industry Tour Graduate Student Leadership Program Mentor (2014)
- American Society of Animal Science Academic Quadrathlon Dairy Coordinator (2013)
- North American Intercollegiate Dairy Challenge Volunteer (2013, 2014)
- Purdue Academic Quadrathlon Dairy Coordinator (2013, 2014)
- Graduate Programs Committee Graduate Student Representative (2013 to 2014)
- Animal Sciences Graduate Student Industry Tour Co-coordinator (2012, 2013)
- Spring Fest Graduate Student Coordinator (2012, 2013)
- Animal Sciences Graduate Student Association President (2011 to 2013)
- Animal Sciences Workshop for Youth Goat Species Facilitator (2011)

Podcasts

Purdue Dairy Digest podcast series (Available at: http://www.ansc.purdue.edu/dd/):

1. Replacement Heifer Research Update (#49)
2. Heat Stress in Dairy Calves (#124)
3. Feed Delivery Methods for Dairy Heifers (#131)
4. Grain Inclusion Levels for Dairy Heifers (#177)
5. Supplementing Biotin to Dairy Cows (#186)
6. Supplementing Niacin to Your Dairy Herd (#189)
7. Folate in Dairy Rations (#193)
8. The Importance of Choline in Dairy Rations (#194)

Grants

2013 Mary S. Rice Grant (Purdue University, College of Agriculture) – $7,250
2012 Mary S. Rice Grant (Purdue University, College of Agriculture) – $9,000

Publications and Presentations

Peer-Reviewed Journal Publications

1. R. C. Schroer, T. D. Nennich, **T. S. Dennis**, M. M. Schutz, S. S. Donkin, and D. Little. 2014. Intake and growth of prepubertal dairy heifers fed reduced-fat dried distillers grains. *Prof. Anim. Sci*. 30:93-98.
2. **T. S. Dennis**, J. E. Tower, and T. D. Nennich. 2012. Effects of feeding hay and baleage to prepubertal dairy heifers during the grower period. *Prof. Anim. Sci*. 28:648-656.
3. **T. S. Dennis**, L. J. Unruh-Snyder, M. K. Neary, and T. D. Nennich. 2012. Effects of co-grazing dairy heifers and goats on forage intake, botanical composition, and dry matter yield of mixed species pastures. *J. of Anim. Sci*. 90:4467-4477.

Peer-Reviewed Extension Publications

1. Nennich, T. D., **T. S. Dennis**, H. Schmitz, and J. E. Tower. 2013. Supplementing Young Grazing Dairy Heifers with Lick Tubs: A Case Study. Purdue Extension AS-610-W.

Selected Peer-Reviewed Abstracts

1. **T. S. Dennis** and T. D. Nennich. 2015. Nutritional Management Strategies to Improve Growth and Feed Efficiency of Prepubertal Dairy Heifers. *Presenting at Midwest Branch ASAS-ADSA Meeting 2015 for ADSA Young Scholar Award.*
2. **T. S. Dennis**, J. E. Tower, A. Mosiman, and T. D. Nennich. 2014. Influence of dietary carbohydrate fractions on growth and development of prepubertal dairy heifers. *Presenting at ASAS-ADSA Joint Annual Meeting 2014. In press.*
3. **T. S. Dennis**, J. E. Tower, H. Schmitz, A. Mosiman, and T. D. Nennich. 2013. Effect of increased dietary grain inclusion on growth performance of prepubertal dairy heifers. *J. Dairy Sci.* 96(E-Supp. 1):717.
4. **T. S. Dennis**, J. E. Tower, H. Schmitz, A. Mosiman, and T. D. Nennich. 2013. Impact of increased dietary grain inclusion on blood metabolites and rumen fermentation characteristics of prepubertal dairy heifers. *J. Dairy Sci.* 96(E-Supp. 1):521.
5. **T. S. Dennis**, J. E. Tower, H. Schmitz, A. Mosiman, and T. D. Nennich. 2013. Impact of providing shade on grazing dairy heifer performance. *J. Dairy Sci.* 96(E-Supp. 1):90.
6. **T. S. Dennis**, J. E. Tower, and T. D. Nennich. 2012. Evaluation of feed delivery methods for transitioning prepubertal dairy heifers to higher forage diets. *J. Dairy Sci.* 95:E-Supp. 2.

Selected Presentations

1. **T. S. Dennis** and T. D. Nennich. "Nutritional Management Strategies to Improve Growth and Feed Efficiency of Prepubertal Dairy Heifers". Midwest Branch Meeting of ASAS and ADSA, March 18, 2015. Invited.
2. **T. S. Dennis.** "Feeding Strategies to Improve Calf Growth". Purdue Extension Dairy Nutrition Update (Elkhart County), December 9, 2014. Invited.

Honors and Awards

2015 American Dairy Science Association Midwest Branch Young Dairy Scholar
2014 Bilsland Dissertation Fellowship Award (Purdue University, Graduate School)
2012 Tri-State Dairy Nutrition Conference Graduate Student Competition (3nd Place)
2010 Tri-State Dairy Nutrition Conference Graduate Student Competition (2nd Place)

Professional Affiliations

American Dairy Science Association (student member)
American Registry of Professional Animal Scientists (student member)
North American Colleges and Teachers of Agriculture (student member)
Indiana Dairy Producer